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CERTAIN RESPONSES OF DOMESTICATED BIRDS TO INFECTION WITH *PLASMODIUM LOPHURAE* COGGESHALL, 1938

by

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INTRODUCTION

In the early part of this century it was believed that malarial parasites resided almost exclusively in the erythrocytes of the vertebrates in which they occurred. Through extensive research during the past twenty-five years, however, it has been established by workers in the field of malariology that in certain species of vertebrates developmental stages of malarial parasites are present within the body tissues and outside the circulating elements of the blood. Such stages have been called exoerythrocytic parasites, a term introduced by James and Tate (1938) for the stage of Plasmodium gallinaceum Brumpt (1935) which they observed outside the erythrocytes in blood-induced infections. Huff and Coulston (1946) classified exoerythrocytic stages on the basis of their origin and the stage of infection in which they were abundant, as follows: (1) Preerythrocytic stages included only those stages arising directly from the sporozoites introduced into the vertebrate body. Huff, Coulston, and Cantrell (1943) proposed the term "cryptozoite" for the first generation of exoerythrocytic stages resulting from sporozoites. Huff and Coulston (1944) proposed the term "metacryptozoites" for all subsequent generations of such stages, derived either from a cryptozoite or from another metacryptozoite, occurring prior to the beginning of the parasitemia. (2) Huff and Coulston (1946) proposed the term "phanerozoite" for the exoerythrocytic stages appearing concomitantly with or later than the parasitemia in sporozoite-induced infections, and for the exoerythrocytic forms appearing in blood-induced infections. Much of the research on exoerythrocytic stages has been

centered on Plasmodium gallinaceum which develops remarkably well in chickens as well as in some of the other common domesticated birds.

A species of malarial parasite discovered a little more recently, and one which lends itself quite favorably to experimental infection of some of the larger domesticated birds, is Plasmodium lophurae Coggeshall (1938), which the describer found in the blood of a Borneo fireback pheasant, Lophurae igniti igniti (Shaw and Nodd), from the New York Zoological Park. This protozoon has been grown successfully in chicks, ducks, geese, turkeys, guinea fowl, and pheasants. Cryptozoites and meta-cryptozoites of P. lophurae have been found in turkeys, chickens, ducks, and guinea fowl and described by Huff, Coulston, Laird, and Porter (1947). Phanerozoites of this species were first observed in the brain capillaries of turkey poults by Becker and Manresa (1950).

Huff and Coulston (1946), and Huff, Coulston, Laird and Porter (1947) reported that phanerozoites of neither P. gallinaceum nor P. lophurae had been demonstrated in ducks, guinea fowl, turkeys or chickens, although pre-erythrocytic stages had been found. Porter (1942), using P. cathemerium, found that this strain produced preerythrocytic stages after passage through mosquitoes. Boyd (1949) suggested that the ability of a species to produce phanerozoites apparently may be lost by repeated passages from host to host by blood inoculation. He further suggested that the ability of a species to produce phanerozoites to any great extent may occur only in infections in which an exceedingly high parasitemia is reached. In addition, he pointed out it is extremely likely that many factors play a part in determining whether, in each particular case, the phanerozoites may be produced.

Such possible factors are species of host or of parasite, immune or susceptible condition of the host, pathogenicity of the malarial strain, and recency of mosquito passage.

Considering that phanerozoites of P. lophurae have never been observed in blood-induced infections of chickens and ducks, but have been found in infected turkeys (Becker and Manresa, 1950; Manresa, 1951), the major problem that suggested itself for the present research was an exhaustive study of the tissues of chickens and ducks, keeping in mind the possibility of locating phanerozoites. Experiments were planned in which 12- and 13-day-old chicks were given intravenous injections of parasitized erythrocytes from young White Pekin ducks. Various tissues removed from the chicks when they were sacrificed, or after they had died from the malarial infection, were examined microscopically.

One minor problem involved the microscopic examination of sections, smears and imprints of tissues and of organs taken from ducks which had been inoculated with turkey brain tissue containing exoerythrocytic stages of P. lophurae. No erythrocytic parasites could be found in previous microscopic examinations of the blood of the turkeys serving as donors.

Similar microscopic examinations were made of sections, smears, and imprints of organs and tissues which had been removed from turkeys that had been inoculated with duck blood which contained infected erythrocytes from a blood-induced infection of P. lophurae.

The problem of second importance was the search for phagocytic cells which are active in the destruction of erythrocytes parasitized with P. lophurae. Cannon and Taliaferro (1931), working with P. cathemerium in the canary, and Taliaferro and Cannon (1936), working with P. brasilianum

in New World monkeys, were the first investigators to study tissues from animals that had been killed at closely spaced intervals throughout the initial infection and the superinfection, and to correlate phagocytosis with the development of the infection. They found the macrophages to be most actively phagocytic in the spleen, and less active in the liver and the bone marrow. Other organs assumed a minor or negative role.

Another problem that was included in the scope of the research was the observation of the daily variation in the percentage of infected erythrocytes of chicks fed different diets but inoculated with comparable numbers of P. lophurae. Brooke (1945), in working with avian malaria parasites, observed that birds which were given deficient diets suffered more severe primary attacks, and showed a greater tendency to relapse with less resistance to superinfection and a higher mortality rate than birds given an adequate diet.

Individual daily weight records were kept of the chicks on the different diets to learn what relationship might exist between the daily weight change and the course of the infection.

The report of Becker, Brodine, and Marousek (1949) on the appearance of eyelid lesions induced with diet in conjunction with infection with P. lophurae suggested a related problem concerning the various diets which were given the different groups of chickens.

Hewitt (1942) reported a correlation between the rise in numbers of infected erythrocytes and the temperature of White Pekin ducks following inoculation with P. lophurae. His work suggested a similar problem for young chicks infected with the same parasite.

REVIEW OF LITERATURE

Historical Background

Plasmodium lophurae, the malarial parasite used in the present experiments

According to Coggeshall (1938), the *Plasmodium* observed in the blood of a Borneo fireback pheasant, *Lophura igniti igniti* (Shaw and Nodd), proved to be transmissible to very young chicks, but usually produced a moderately severe disease which did not generally end fatally. Morphologically and pathogenically it appeared to differ from all other known species of *Plasmodium* commonly found in birds, hence was given the status of a new species. Because it was obtained originally from a fireback pheasant, the name *Plasmodium lophurae* was proposed for it. Morphological characteristics of *P. lophurae* were observed by Coggeshall (1938) and include the following features:

1. All stages in the development of the parasite are sharply defined in the cytoplasm of the red blood cells after Giemsa staining with the trophozoites found either at the side of or at the end of the red blood cell nucleus. Multiple invasion of the red blood cells by the young trophozoites occurs frequently, but enlargement of the red blood cells does not occur when they are invaded by the parasites. Rarely is there any displacement of the cell nucleus by the invading parasite.

2. The pigment, present in all stages of development of the parasite, is dark brown, except in the gametocytes, and may be diffusely scattered or may appear as discrete granules. In the gametocytes, the pigment is in the

form of discrete granules which are refractile in appearance. Merozoites, counted in the segmenting forms before sporulation occurred, varied in number between eight and eighteen.

3. The microgametocytes usually surround the red blood cell nucleus. However, the cytoplasm of the microgametocytes is so faintly stained that it frequently appears transparent. Pigment of the microgametocytes appears as separate, refractile granules, but the chromatin granules are diffusely scattered.

4. Cytoplasm of the macrogametocytes stains dark blue with Giemsa, and the chromatin is usually more compact than that of the microgametocyte. Frequently the chromatin appears in a band form. Pigment granules of the macrogametocytes are distinct, refractile, and scattered throughout the parasite.

Concerning the pathogenicity of P. lophurae, Coggeshall (1938) made the following observations:

Domesticated birds which were found to be susceptible to P. lophurae included Rhode Island Reds, White Leghorns and Brahmas. Transfer to canaries was observed in only two of the birds tested, and in both cases the infections were extremely mild, with a parasite count of only nine parasites per 10,000 red blood cells, and lasted only four days. Pheasants were susceptible to the parasite. Two white pheasants had microscopically visible parasites in their blood for periods up to six months after the original inoculation. Pigeons were found to be nonsusceptible to P. lophurae.

The acute phase of the infection following blood inoculations for birds which were susceptible to P. lophurae usually lasted about seven

days, but was sometimes found to last as long as two weeks. After the disappearance of the circulating parasites, blood was subinoculated into normal chickens, but was not found to be infectious to them. Transfer of infection from adult to young chickens was found to be possible only when parasites were visible in the blood. In adult chickens and pullets there were only mild infections by transfer of infected blood. The maximum parasite count was found to be only one parasite during a search of five minutes. Maximum length of infections was but two days.

Thirty female Aedes aegypti mosquitoes were permitted to feed on a chick that had numerous male and female gametocytes present in its blood, then six days later fifteen of these mosquitoes were dissected and examined for the presence of oocysts. Of these fifteen, nine had from two to fifteen oocysts present on the wall of the stomach, while six of the fifteen were negative for the presence of oocysts. None of the Culex pipiens mosquitoes tested became infected. One mosquito had a few small oocysts present in the stomach wall, but no sporozoites could be demonstrated in the salivary glands. Anopheles quadrimaculatus and Anopheles punctipennis mosquitoes were tested to determine whether or not they might become infected, but none was found to do so.

Taliaferro and Taliaferro (1940) described the infections in young chicks with P. lophurae as characterized by an acute rise in the numbers of parasites, terminated by a sharp crisis and followed within a few days by a latency ordinarily not interrupted by relapses. However, they demonstrated that for periods of at least four months the parasites could be observed in the so-called "recovered" chicks by the subinoculation of large quantities of their blood into normal chicks to produce an infection.

Terzian (1941b) described the gross pathology of chicks infected with P. lophurae as typical for that of the liver, spleen, and bone marrow of other species of avian malaria. He described the lack of relationship between splenomegaly and the degree of parasitemia as being shown by the lack of correlation between spleen size and the parasite densities of 19 birds killed at various times after the sixth day of the infection.

Taliaferro and Taliaferro (1950) found that initial infections of P. gallinaceum and P. lophurae in chickens had the same length asexual cycle of 36 hours, but differed markedly in relation to synchronism of the cycle, rate of reproduction, and the survival and death of parasites. Evidences of acquired immunity appeared progressively against P. gallinaceum, but suddenly at the peak of the infection against P. lophurae.

To test the infectivity of P. lophurae, McGhee (1950) injected erythrocytes from 16 species of animals representing three different classes into the blood stream of chick embryos which had been infected with this avian malarial parasite. Of the 16 species tested, parasites were observed in the erythrocytes of the human being, the rabbit, pig and mouse. Cells of the rabbit and pig disintegrated rapidly so it was impossible to trace the actual development for more than a period of ten hours. The human erythrocytes retained their characteristic morphology for a longer period of time, but exhibited a rather high degree of resistance to infection. Erythrocytes of the white mouse were well preserved and became infected very readily. It was observed that only erythrocytes in which the potassium level was high were subject to infection.

McGhee (1951) was able to infect infant mice with intravenous inoculations of chick embryo blood that was heavily infected with P. lophurae

so that the mice reacted with a low-grade parasitemia. By alternating embryo passages with those of mouse passages the course of infection in the mouse became modified so that greater peaks of parasite numbers were attained and the period of patent parasitemia was increased. After four such passages it was possible to pass the infection from mouse to mouse without any apparent lessening of viability on the part of the parasite. The establishing of P. lophurae in the mouse was accompanied by distinct morphological changes, with a tendency on the part of the parasite toward assuming the characteristics seen in avian cell infections.

Coffin (1951), in his work with ducklings immunized against P. lophurae by inoculations of formaldehyde-killed tubercle baccili, showed that the ducks had increased their resistance to blood-induced infection with P. lophurae. Chickens, weighing initially 2.5 kg., immunized with P. lophurae in a similar emulsion and twice infected with P. lophurae showed a greatly increased resistance to blood-induced infection with P. gallinaceum, as compared to normal unimmunized chickens, or chickens infected twice with P. lophurae.

Various workers have attempted but failed to produce severe infections of P. lophurae in older chickens by such means as exposing them to a lower temperature, maintaining them on a diet which was low in vitamins of the B_2 group, or treating them daily with phenylhydrazine hydrochloride. Trager (1941), however, was able to produce severe infections in chicks whose ages varied from four to nine weeks by inoculating them with large doses of P. lophurae parasites, and in addition, injecting doses of diluted carbon ink into the ventral portion of the posterior peritoneal cavity. Doses of 5 cc. per 100 grams of body weight were given the chicks the day

before their inoculation with malarial parasites, and for several days thereafter. The course of the malarial infection in the chicks inoculated in this manner resembled that seen in very young chicks, suggesting that the ink treatment interfered with the metabolism responsible for the age immunity which is observed among untreated, older chicks.

Rigdon (1944) inoculated two-weeks'-old White Pekin ducklings with blood taken from passage ducks that had P. lophurae infections in an effort to study the acute pathological lesions which might be produced by this malarial parasite. His efforts indicated that no pathological lesions could be observed in birds which were killed the second day following inoculation, but from the fourth day until the fifteenth, the basic lesions which occurred in ducks suffering from P. lophurae infection were similar to those he found in P. falciparum infection in a child, and those of P. knowlesi in Macacus rhesus monkeys. The following material describes the lesions which Rigdon observed:

The liver was definitely pigmented the fourth day after inoculation. The amount of pigment increased up to the fifteenth day, after which no further additional pigmentation was observed. The lungs and the femoral bone marrow became progressively pigmented as the infection developed. In the lungs of some of the birds there were observed focal, reddish-blue areas resembling hemorrhages. Often, there was an acute fibrinous pericarditis accompanied by hemorrhages in the epicardium. Progressively, the myocardium became more and more pale and flabby during the acute phase of the infection. Among the birds which survived the infection, the color of the myocardium gradually returned to its normal color after the infection had subsided. The focal areas of degeneration observed in the myocardium

of some of the ducks became infiltrated with red blood cells and leucocytes. No lesions were observed in the brain regions. Minor pigmentation was noted in some of the skeletal muscles, the skin and parenchymal tissues.

In the microscopic examination of the various tissues, Rigdon (1944) observed a few small granules of malarial pigment to be present the second day of the infection in the spleen. As the infection progressed, granules of yellow pigment increased in amounts in the spleen, and with their increase, these granules appeared to adhere to one another to produce small masses of pigment. During the first six or seven days of the disease, the aggregates were distributed rather evenly throughout the pulp of the spleen. However, after this period the masses of pigment appeared to accumulate around the walls of the larger sinuses of the spleen. Hyperplasia of the blood forming tissue of the spleen was most conspicuous in the walls of the larger, venous sinuses. Masses of these cells seemed to extend into the lumina of the sinuses and a single layer of endothelial cells sometimes covered these groups of reticular-like cells. Similar effects and conditions were observed in the tissues making up the liver and the kidneys. In the liver there was a progressive increase in the amount of phagocytosis by the Kupffer cells which could be observed from the second day of the infection for a period of about 13 or 14 days. Both the malarial pigment and cellular debris could be seen in the cytoplasm of these phagocytic cells. After the third day the cytoplasm of the hepatic cells of most of the birds became somewhat vacuolated.

Degeneration in the epithelial cells of the renal tubules occurred in varying degrees. Masses of blood forming cells appeared in the medulla region of the kidneys. These were found in the walls of the blood sinuses

and projected into the lumina. In some of the ducks observed, the lumina of many of the large blood vessels were filled with these thrombus-like masses.

After the second day of the infection, Rigdon (1944) observed granules and small masses of malarial pigment. The pigment appeared to be held by the circulating phagocytes in the lumina of the capillaries. As the disease progressed, it was difficult to find phagocytic cells in the larger masses of pigment. Phagocytosis of pigment by the endothelial cells in the blood vessels appeared to be nonexistent, as the pigment seemed to be present mostly in the circulating phagocytic leucocytes.

After the fifth day of the infection, groups of mononuclear cells could be found in the lumina of some of the large blood vessels. They were similar to those found in the walls of the blood vessels of the liver, the spleen and the kidney, and appeared to be hemopoietic cells. At times, granular leucocytes, red blood cells and malarial pigment could be found in the areas with the mononuclear cells.

From the observations made by Rigdon in 1944 on the pathological lesions in the ducks which were killed within the first fifteen days of the infection with P. lophurae it has been suggested that the rapidly progressing anemia plays a significant part in producing the death of the birds which have been tested. The anemia was produced as a result of the rapid destruction of the red blood cells by the malarial parasites. The extensive hyperplasia observed of the blood forming tissues was an indication of the reaction of the host to the anemia.

Laird (1941) found that three species of mosquitoes, Culex restuans, Aedes atropalpus, and Aedes albopictus were all susceptible to P. lophurae.

Aedes aegypti and Culex pipiens were not infected. The presence of apparently mature sporozoites in the salivary glands of the mosquito host did not always indicate the ability to infect a susceptible animal.

Exoerythrocytic stages of various plasmodia

Recent research has greatly enlarged as well as advanced our knowledge of the asexual cycle of the malarial parasites, but some of the early malariologists recognized the possibility that schizogony might occur elsewhere in the body than in the erythrocytes. Golgi (1893) observed all stages of the schizogonic cycle, often without visible signs of digestion, within the phagocytes of the internal organs, and suggested that progressive development might occur in such cells which would thus protect the parasites against any drug treatment used against them. Grassi (1900), after observing the morphological differences between the nucleus of sporozoites and young trophozoites, suggested on the basis of this evidence that an intermediate cycle of some peculiar character occurred in vertebrates. In 1902, however, the question of sporozoite development became a closed issue insofar as most workers were concerned, following the description and illustrations of Schaudinn (1902) showing the entry of sporozoites into red blood cells. Nevertheless, other workers, such as Carducci (1905), as was pointed out by Porter and Huff (1940), suggested that the parasites survived in some of the internal organs. In 1910 Celli postulated the existence of especially resistant stages.

Attempts made by numerous workers, among them Yorke and MacFie (1924), Yorke (1931), Boyd and Stratman-Thomas (1934), and others, to reproduce the phenomenon of the direct penetration of the erythrocytes by the

sporozoites, as claimed by Schaudinn (1902), resulted only in failure.

James (1931), in his observations of malaria in England, was led to believe that intermediate stages existed between the sporozoite stages and the erythrocytic stages. He postulated that the sporozoites underwent some special development in the connective tissue cells or in the endothelium in his explanation for the difference in response to quinine of the mosquito-induced malaria and the malaria following blood inoculation. Some indirect evidence in favor of James' view was presented when there was found to be a period of non-apparent parasitism during which interval the blood of the host which had received sporozoites proved to be uninfective when subinoculated. The following list includes some who have observed this phenomenon: Boyd and Stratman-Thomas (1934); Kikuth (1935); Warren and Coggeshall (1937); Kikuth and Mudrow (1938); Missiroli (1937); Ciuca, Ballif, Chelarescu, Isanos, and Glaser (1937); Boyd and Matthews (1939); Henry (1939); and Coulston, Cantrell, and Huff (1945). By implanting tissues from hosts suffering from avian malaria into other susceptible birds, Warren and Coggeshall (1937) proved that during this so-called "negative phase" of the blood, P. cathemerium parasites actually were present in the spleen, bone marrow, and the area in which the sporozoites had been inoculated. Results of these workers were confirmed and extended to other species as well as to other organs of the host by Kikuth and Mudrow (1938, 1939), De Court and Schneider (1938) and Coulston, Cantrell, and Huff (1945).

Descriptions of stages of avian malarial parasites given by Anschutz (1909, 1910) and Ben-Harel (1923) can be recognized now as descriptions of actual exoerythrocytic stages of P. circumflexum and P. elongatum,

respectively, although these workers did not realize them to be such. Huff (1930) noted that stages of P. elongatum were abundant in hemopoietic tissue and in the young red cells. Raffaele (1934a) made this same observation, and also noted that frequently there was an absence of pigment in the parasites. Raffaele (1934b) further noted that P. elongatum developed in a great variety of cells, many of which he incorrectly placed in the reticulo-endothelial system.

Huff and Bloom (1935) described schizogonic forms of P. elongatum in all cells of the lymphoid and myeloid series including monocytes, plasma cells, thrombocytes, macrophages, hemacytoblasts, erythroblasts, erythrocytes, lymphocytes and myelocytes. However, even though P. elongatum parasites were found in the various blood cells and blood forming cells, these workers observed that the overwhelming majority of the parasites occurred in members of the red blood corpuscle series. In their work of 1935, Huff and Bloom suggested it is probably that the occurrence of the parasites in the various kinds of phagocytes, including the macrophages, monocytes and granulocytes, may indicate that the parasites in these cells are destined for destruction. This was supported by the presence of living as well as degenerating parasites observed in these cell types. Occasionally the parasites were observed in the plasma cells, which are of the non-phagocytic type. Since degenerating parasites were not found in these cells, Huff and Bloom (1935) concluded that plasma cells offered a suitable medium for the progressive development of P. elongatum parasites.

Raffaele (1936) demonstrated that large, non-pigmented schizonts could be found in the leucocyte or endothelial cells of canaries after they had been heavily inoculated with sporozoites of P. relictum, then

sacrificed on the first day of patency.

James and Tate (1937b) demonstrated the development of stages of P. lophurae as well as growth of other avian malarial species in connective tissue cells in vitro. In the species tested the exoerythrocytic stages developed almost exclusively in macrophages and capillary endothelium.

James and Tate (1938) described the schizogony of P. gallinaceum which they observed occurring in the monocytes and cells of the reticulo-endothelial system as "exoerythrocytic schizogony" to distinguish it from the schizogony which occurs in the red corpuscles. They further noted that the exoerythrocytic schizonts were characterized by the absence of malarial pigment, their large size and by the presence of numerous merozoites numbering up to 60 or even more. These workers found that the exoerythrocytic schizogony was not confined to the leucocytes of the blood stream but could develop in the fixed endothelial cells of organs such as the brain, lungs, liver and spleen, with the brain being the first organ in which the exoerythrocytic schizogony was observed. They found these forms in the capillaries of the brain as soon as the parasitized red blood cells were present in the peripheral blood circulation, but not before that time. In the brain, rows of schizogonts were developed in some instances, and ultimately they occluded most of the capillaries of the brain. The exoerythrocytic stages of P. gallinaceum occurred in birds which were inoculated either with infected blood or with sporozoites.

James and Tate (1938) further described the growth of the exoerythrocytic schizont. They noted that the host cells became greatly distended with the nucleus being pushed to one side and finally compressed. Eventually there was disintegration of the host nucleus, and the host cell

became completely filled with a large mass of merozoites around the remains of the old cytoplasm. In dry smears the mature schizont was found to be completely devoid of the host cell. When stained properly, the schizont had a reddish tinge with the merozoites enclosed in a sac-like membrane. The schizont was limited in size by the type or size of the host cell in which it developed.

Workers using other species of Plasmodium recognized the exoerythrocytic stages of these forms. Kikuth and Mudrow (1937) demonstrated them in P. cathemerium; and Coulston (1939), and Manwell and Goldstein (1938, 1939) observed them in P. circumflexum. The only species of Plasmodium parasitizing lizards as yet demonstrated to possess exoerythrocytic schizogony is P. mexicanum observed by Thompson and Huff (1944).

Wolfson (1940a) found that apparently exoerythrocytic schizogony of the wood thrush strain of P. cathemerium could be temporarily prevented from developing in canaries by the passage of the infection through ducks. Canaries infected with the wood thrush strain of P. cathemerium in which exoerythrocytic schizogony seemed to be prevented from developing by passage of the infection through ducks and pigeons, died, on an average, about 17 days after inoculation. Generally they lived about as long after inoculation as do canaries in which exoerythrocytic schizogony is observed upon autopsy. Infarcts and enlargement of the spleen as well as hemorrhages in the membrane of the brain among canaries found dead in their cages, occurred much more frequently in those which showed exoerythrocytic schizogony than in those in which such schizogony could not be demonstrated. Thus the wood thrush strain of P. cathemerium in ducks has not been found associated with exoerythrocytic schizogony, whereas such schizogony is

characteristic of canaries infected with this strain of Plasmodium.

Dobler (1941) reported exoerythrocytic schizogony associated with the matinal strain of P. relictum in canaries after the strain had passed through two ducks. Two canaries had been inoculated with blood from the same duck, and exoerythrocytic bodies appeared in the second transfer from these birds. The course of the infection of the matinal strain of P. relictum and that of the exoerythrocytic strain were similar, with the exception that the birds which were infected with the exoerythrocytic strain became ill and died approximately 10.4 days after inoculation. Parasite count in the peripheral blood was negligible at this time. The average length of life for a canary infected with the wood thrush strain of P. relictum was 10.4 days compared with that of one infected with P. cathemerium which was 15 days. Spleens of birds inoculated with P. cathemerium were slightly larger and showed more infarcts than those infected with P. relictum.

Attempts made by Dobler (1941) to produce exoerythrocytic schizogony by inoculating the Mexican strain of P. relictum and the Hartman-Hewitt strain of P. cathemerium into ducks and then into canaries, were negative.

According to Terzian (1941b), examination of smears of the liver, spleen, bone marrow, brain and lungs of 25 young Rhode Island Red and White Leghorn chicks infected with P. lophurae failed to disclose the presence of exoerythrocytic bodies. He indicated, however, that since a given strain may show such bodies only in occasional infections, there is always the possibility that the parasites did not happen to be undergoing such a cycle at the time the birds were examined.

Porter (1942), using P. cathemerium as the parasite and the canary as the host, observed the distribution of exoerythrocytic schizonts in sporozoite-induced infections. Exoerythrocytic schizonts occurred almost exclusively in macrophages of the liver, spleen and bone marrow during the earlier stages of infection. Late in the infection the parasites became abundant in the ordinary endothelium and capillaries throughout the body. Numerous parasites also occurred in the intravascular accumulations of macrophages in all organs. It has been suggested that the early exoerythrocytic forms found in the macrophages came about as a result of sporozoite development, whereas the later stages found in the macrophages, endothelial cells and the capillaries arose as a result of certain physiological changes occurring in the host, which enabled the erythrocytic merozoites to develop in an exoerythrocytic manner. Regardless of the explanation, it seems probable that early and late schizogony involved different processes.

Porter (1942) looked further for exoerythrocytic schizonts in dry smears of tissues from birds infected with four strains of P. relictum, and in birds infected with the bluebird strain of 9J of P. hexamerium, the Sergent (4A) strain of P. rouxi, and with the Kikuth strain (60) of P. circumflexum, but none were found. Neither were exoerythrocytic forms observed during blood passage in eight strains of P. cathemerium. Exoerythrocytic forms were found, however, in birds infected with five virulent strains of P. cathemerium, including the 3T Wolfson. The other strains were less pathogenic than these five. In two of the less virulent strains, the Hartman and the Hackett, exoerythrocytic schizogony appeared after the parasites had been passaged through mosquitoes. The other strains were not tested.

Huff and Coulston (1944) working with P. gallinaceum observed that sporozoites entered cells of the lymphoid-macrophage system within 30 minutes after inoculation into the skin of young White Leghorn chicks. These cells served as hosts for all stages of the parasites of the first generation of preerythrocytes (the cryptozoites of Huff, Coulston and Cantrell of 1943). This included the forms which were still recognizable as sporozoites, to large schizonts and segmenters. Sporozoites were found in heterophil leucocytes as late as six hours following inoculation. Only a few of them showed any appreciable development after 24 hours and none were found to complete their development in this type of cell. Sporozoites were not found in the intercellular spaces after six hours. The first of cryptozoic generation required about 42 hours to develop with a range of 36 to 48 hours, whether the parasites grew locally at the site of inoculation or in the spleen. The second generation (first generation of metacryptozoites) was found in cells of the lymphoid-macrophage system and in endothelial cells. This generation underwent segmentation at 70 to 84 hours. These parasites were similar to the cryptozoites. After 60 hours numerous metacryptozoites could be observed, especially in the inoculation sites of the skin following intradermal inoculation, and in the spleen, heart, kidney and brain in the order named. At the same time, sparse erythrocytic parasites were seen at 90 hours after which they gradually increased in numbers up to about six days. Then followed a great increase in numbers of erythrocytic parasites termed a "flooding effect". Five days after inoculation of sporozoites or of the bites of mosquitoes, two types of segmenters were abundant. One of these consisted of small numbers

of large-sized merozoites of the cryptozoic type (macromerozoites), while the other consisted of large numbers of small-sized tightly packed merozoites of the type seen in blood-induced infections (micromerozoites).

Tonkin and Hawking (1947) described the growth of exoerythrocytic stages of P. lophurae in cultures made from the spleen of a turkey inoculated five days previously with sporozoites from Aedes albopictus mosquitoes. The parasites were similar morphologically to those of P. gallinaceum from cultures.

Huff, Coulston, Laird and Porter (1947) observed that the preerythrocytic stages of P. lophurae resembled closely the corresponding stages of P. gallinaceum. These stages were found in cells of the lymphoid-macrophage system, in fibroblasts and in heterophil granulocytes but they did not attain maturity in the granulocytes. Degenerating preerythrocytic stages were observed in all four hosts tested, including the guinea fowl, chicken, turkey and duck. They were least abnormal in appearance in the guinea fowl, most abnormal in the ducks and intermediate between these two in turkeys and chicks.

Dubin (1947) noted two forms resembling exoerythrocytic stages of P. gallinaceum in a four-day-old culture of bone marrow taken six days after a patient had been bitten by mosquitoes infected with P. vivax. The tissue culture method was used on the possibility that if exoerythrocytic forms were present in the specimens, they might multiply in vitro and thus be detected more easily.

Dubin (1948), in his search further for exoerythrocytic forms in human malaria, made tissue cultures from the bone marrow of patients having sporozoite-induced infections, both in the prepatent period as well as

after the development of the parasitemia. This was done in P. falciparum infection in six instances, and in twenty instances of P. vivax infections. No exoerythrocytic forms were observed in the cultures, however.

Huff and Coulston (1948) found no evidence of preerythrocytic stages of P. cynomolgi in organs of the monkey, Macaca mulatta, inoculated with sporozoites from 11 to 75 infected Anopheles quadrimaculatus mosquitoes. Likewise, the microscopic search for preerythrocytic stages of P. vivax parasites in organs of five species of monkeys failed to show evidence of their presence.

In his paper concerning exoerythrocytic forms of malarial parasites, Huff (1948a) pointed out that exoerythrocytic stages of malarial parasites are well known in only certain species of Plasmodium parasitizing birds and in one species parasitizing lizards. In one of these, P. elongatum, described by Huff and Bloom (1935) almost all types of cells found in the blood and in the blood-forming cells of the host could be parasitized, but the cells of the erythroblastic series seem to be preferred. In all of the other species of avian malaria which have been adequately studied, except P. elongatum, the cells of the lymphoid-macrophage seem to be preferred, according to the observations of Huff (1948b), although true endothelium and some other types of cells may be parasitized. In the species of P. gallinaceum, P. relictum, P. cathemerium and P. lophurae, sporozoites required a period of development in macrophages and in related kinds of cells before they invaded the erythrocytes. Collectively, then, these stages were called "preerythrocytic stages", with the first generation following inoculation by sporozoites being the "cryptozoites" and the subsequent generations the "metacryptozoites".

Lewert (1948) using blood-infected, quinine-treated chicks, analyzed and compared exoerythrocytic infections of the central nervous system by P. gallinaceum with exoerythrocytic infections of other organs and tissues. He found the exoerythrocytic parasites in all regions of the brain of infected chicks, with the number of parasites per unit volume varying in different regions. This difference in parasitic density among the different regions of the brain showed a similar trend in the different infected individuals tested. However, this difference appeared to be directly related only to the differences in capillary density. Early stages of the exoerythrocytic infection of the brain capillaries were characterized by the great predominance of young schizonts but the later and terminal stages of the infection had a greater proportion of older, mature schizonts. Primary parasitemia was suppressed by quinine therapy, but uninuclear erythrocytic parasites reappeared when the exoerythrocytic parasites became well established. These "ring" forms were not accompanied by older erythrocytic stages and were more frequent when the density of the exoerythrocytic parasites was high.

Lewert (1948) further observed that capillary occlusion was due to intraendothelial growth of parasites, and, to a lesser extent, to emboli formed by the extracellular schizonts found in great numbers only in dense infections. There was no indication that the infection of the capillary endothelium was preceded by any "endothelial stickiness", even though once the infection was established the increase in adhesiveness of the capillaries for carbon particles was proportional to the extent of capillary invasion. Prior to the appearance of parasites in the capillary endothelium of organs and tissues, the capillary endothelium of the nervous

system was invaded. Before the appearance of intraendothelial forms in the central nervous system, Lewert found no reservoir of exoerythrocytic parasites. The organs and tissues having long, relatively unbranched capillaries appeared to favor infestation over organs and tissues having a richly anastomatic circulation.

Lewert (1950) noted that great difficulty was encountered in the direct observation of exoerythrocytic stages of P. gallinaceum in cultures. In only one case was it possible to observe living parasites. However, in the chicks inoculated with material from the cultures, the infections produced were essentially if not entirely exoerythrocytic. Prior to the death of the birds examined, no erythrocytes infected with the pigmented parasites normal to Plasmodia could be found in the peripheral circulation. The liver and spleen lacked the accumulations of pigment normally present in such infections. Gross indications of tissue damage were conspicuous in the birds infected from cultures. The pericardial cavity was always distended with large accumulations of fluid. The livers were enlarged and the surfaces spotted with both small and large hemorrhages. Spleens were greatly enlarged and of a dark purple color.

Sergent (1949) pointed out that exoerythrocytic forms of P. relictum had been observed in all the main organs of the canaries which he had used as hosts for this parasite.

Greenberg, Trembly and Coatney (1951) reported that when they passaged four independent series of phanerozoiteless strains of P. gallinaceum through young chicks, there was a marked change in the behavior characteristics of the strains after 15 to 42 different blood transfers. At first, the rate of survival of the chicks used in the experiments was

very high, but with an increase in the number of transfers, the chicks died in greater numbers, until finally, 78 percent of the chicks that survived the parasitemia subsequently died of phanerozoite infections. These workers suggested that the ability of the parasite to invade and to reproduce in the fixed tissues was maintained through mosquito passage.

In a paper by Trembly, Greenberg and Coatney (1951a) it was reported that of 290 chicks which had been inoculated with varying numbers of sporozoites of blood-passaged Bl strain of P. gallinaceum, 271 became infected but the pattern of infection produced was not affected by the number of sporozoites inoculated into them. Twenty-seven percent of the chicks died of heavy exoerythrocytic infections with no normal pigmented erythrocytic parasites detected in the exoerythrocytic pattern. Just one chick developed a normal parasitemia which later became chronic. Sporozoite passages from this chronic infection resulted in a predominance of exoerythrocytic infections. A single oocyst inoculation derived from this chronic infection was successful and a chronic infection resulted. This infection was further passaged by a single oocyst. As a result of oocyst transfer, the strain was modified so that normal parasitemia with gametocytes was produced. Inoculation of oocysts from blood-passaged Bl strain infection produced only an exoerythrocytic infection. Results indicated that with this species of malarial parasite the parasitism of the blood must be preceded by a tissue parasitism. It is considered to be unwarranted to assume, however, that such is the case with all malarial parasites. Furthermore, the kinds of tissue parasitism might possibly be very different in the various species of parasites.

Coulston and Huff (1948) observed the effects of various antimalarial

drugs upon the preerythrocytic stages of P. gallinaceum and found that cryptozoites were able to grow in the macrophages of the skin in chickens which had been given sulfadiazine, sulfamerazine, sulfapyrazine, metanilamide, and naphthoquinone in dosages which were several times as great as was necessary to prevent parasitemia. The majority of these cryptozoites thus produced appeared to be normal. However, in some of the chicks treated with sulfadiazine, naphthoquinone, and to a lesser extent, metanilamide, there were marked damages of the cryptozoites. In all the drugs tested, the effects on the metacryptozoites were rather pronounced. Large vacuoles appeared in these stages, the numbers of merozoites produced was reduced and there was a delay in their development.

Huff (1951) searched for preerythrocytic stages of P. relictum, P. cathemerium and P. gallinaceum in new parasite-host combinations. No definite relationship was established between the ability of sporozoites to develop in tissues of hosts and the systematic classification of the hosts. In ducks, degenerating preerythrocytic stages were observed along with normal ones in the tissues of ducks which had been inoculated with sporozoites of either P. relictum or P. gallinaceum. Preerythrocytic but no erythrocytic stages were found of P. relictum in turkeys, pheasants and ducks; of P. cathemerium in ducks; and of P. gallinaceum in turkeys and canaries. An intensive search of the tissues of chicks that had been given intravenous inoculations of the sporozoites from 100 to 300 mosquitoes infected with P. gallinaceum showed the presence of first generation (cryptozoic) stages in the spleen only. After the first schizogony, all the organs with the exception of bone marrow were found to be infected with preerythrocytic stages.

Phagocytosis occurring in malarial infections

According to Boyd (1949) the early investigators who studied malarial infections recognized the importance of phagocytosis which they observed. It has been demonstrated that macrophages phagocytose parasitized erythrocytes, isolated malarial parasites, malaria pigment and other debris from malaria. However, opinions have differed as to whether phagocytosis plays a primary or a secondary role during the infection.

The first investigators to study tissues from animals killed at closely spaced intervals throughout the initial infections and superinfections, and to correlate phagocytosis with the development of the infection and with innate and acquired immunity were Cannon and Taliaferro (1931) using P. cathemerium in the canary, and Taliaferro and Cannon (1936) working with P. brasilianum in New World monkeys. They observed that the death of the parasites during innate immunity was correlated with a sluggish phagocytosis of parasites during all stages of their development, by the macrophages of the spleen, liver, and bone marrow. At the time of the crisis, the increased disappearance of the parasites from the blood was associated with the retention of, and possibly agglutination of, the parasites in the Billroth cords of the spleen. Apparently these parasites were soon opsonized and taken up by the macrophages. It would appear that the main difference between innate and acquired immunity was the difference in the rate of phagocytosis by the macrophages of the spleen, liver and bone marrow.

Cannon and Taliaferro (1931), working with canaries which were infected with P. cathemerium, noted that the entire parasite-erythrocyte

combination, rather than merely the isolated merozoite, was phagocytosed in all stages of its development. In addition, they found that phagocytosis by macrophages occurred as early as four hours following inoculation, but rarely earlier than this period, except to a very limited extent. After the four-hour period was passed, phagocytosis occurred constantly during the entire acute stage lasting eight to ten days following inoculation with the Plasmodium. Eighteen hours after inoculation, mesenchyme tissues seemed to be activated. The peak of the activation of the mesenchyme appeared between the eighth and tenth day of the infection, coincidently with the crisis of the infection during which time the majority of the parasites disappeared from the peripheral circulation. This activation was evidenced in the liver by the marked swelling of the Kupffer cells which showed pronounced phagocytosis. There was also a great increase in the number of mononuclear cells and in the numerous mitotic figures, together with a disorientation of the liver cords. In the spleen there appeared to be a diffuse hyperplasia of the lymphoid cells and an increase in the number of macrophages which contained large masses of phagocytosed pigment. Phagocytosis of the parasite-erythrocyte combination appeared to be limited chiefly to the macrophages of the spleen and liver and to a much less degree in other organs. Phagocytosis by the endothelial cells lining the blood vessels was not observed.

Taliaferro and Cannon (1936), in experimental studies with the malarial parasite, P. brasilianum, of the Panamanian monkey, observed that the parasites became highly concentrated in the spleen and liver at the time of the crisis. Free parasites were numerous in the splenic pulp even though the peripheral blood showed just a few parasites present in a

thick blood smear. At times the liver showed a slight concentration of parasites. After a day or two of this concentrating process, the macrophages suddenly became very active and cleared the spleen and liver of their accumulated organisms, so that oftentimes no parasites could be found in the spleen and liver although some were present in the blood. They found, too, that the reticular macrophages of the splenic pulp and the Kupffer cells of the liver were greatly swollen and contained large masses of coalesced pigment as well as parasites in all stages of digestion. In addition, they observed that when just a few parasites were inoculated into the test animal, activity of the phagocytic cells developed at a slower rate than when large numbers were used.

Taliaferro and Mulligan (1937) reviewed the work of Taliaferro and Cannon (1936) on the cellular reactions during primary infection with P. brasilianum in Panamanian monkeys as well as the earlier observation made by Cannon and Taliaferro (1931) on canaries infected with P. cathemerium, and noted that these workers emphasized in particular the importance of macrophage activity in the defense of the body against these infections. Although the work of Taliaferro and Mulligan (1937) followed, and in some ways supplemented, these earlier studies, the significance of this more recent work lies in the overwhelming evidence which demonstrated the importance of lymphoid hyperplasia as a defense mechanism against malaria. Evidence of this was seen in the transformation of the lymphocytes and monocytes into macrophages. Most of the macrophages developed from medium-sized lymphocytes, but some developed from large lymphocytes, and others from small lymphocytes. They referred to the early work of Maximow (1902 and 1928) in which it was demonstrated that lymphocytes and

monocytes migrated from blood vessels into areas of local inflammation, and were transformed rapidly into macrophages.

Taliaferro and Mulligan (1937) observed that in rather mild infections caused by P. cynomolgi in Rhesus monkeys, the death of the parasites was correlated with the phagocytic activity of the macrophages, especially of the spleen, and to a less degree of the liver and bone marrow. Pigmented macrophages could always be found in the lungs but most of them were intravascular, and the few which appeared to be extravascular were probably some which had migrated from the vessels.

Gingrich (1941b) gave canaries daily intravenous inoculations consisting of 1 cc. of 50 percent suspension of washed foreign avian red blood cells which had been removed from canaries, pigeons and ducks. Inoculations were given for periods of three days in an attempt to block the macrophage system. This blocking off treatment had no effect, however, on the daily rate of increase in the numbers of parasites of P. cathemerium during the acute rise of the infection. Thus it appeared that phagocytosis was not the primary factor involved in determining the death rate of approximately two-thirds of the parasites in the birds which showed a natural immunity to this infection.

Hewitt (1942), in describing the malarial parasite P. lophurae and illustrating in color the stages found in the red blood cells of ducks infected with this organism, based his work on observations made on the course of the infection of about 300 ducks of varying ages, weights and breeds. Of thirty young ducks which had been inoculated with the parasite when they were from four to seven weeks old, more than 75 percent died from what appeared to be the effects of the parasite. Of the others,

250 mature ducks, infections in approximately 10 to 15 percent terminated fatally. In the ducks inoculated with P. lophurae and tested, Hewitt found that the number of heterophiles containing the ellipsoidal rods showed a relative increase when large numbers of parasites were present, and that toward the end of the acute period, or the period just preceding death, some of the heterophiles appeared to show some evidence of having phagocytosed malarial parasites and malaria pigment. Phagocytic cells larger than monocytes and containing great numbers of vacuoles in the cytoplasm, sometimes appeared in the peripheral blood of ducks that were heavily infected with P. lophurae. In nearly every case when these cells appeared, Hewitt noted that they contained evidence of having phagocytosed malarial pigment.

Zuckerman (1945) in studying the phagocytic activity of tissue culture macrophages of chicks against P. lophurae observed that in the presence of normal serum, or of serum taken during the acute initial infection, the macrophages sometimes phagocytosed the free pigment and other debris. In the presence of serum taken from the birds after several superinfections, but not after the reduction of the initial infection to latency, phagocytosis was stimulated, which indicated that infected and uninfected red cells were opsonized.

Alvarez (1952) reported that phagocytes which were introduced into the peritoneal cavity of chicks were capable of engulfing P. gallinaceum parasites placed in contact with them artificially. The parasites infecting the red blood cells were killed or inactivated by the phagocytes after phagocytosis had occurred.

Infectivity of malarial parasites

Coulston, Cantrell and Huff (1945) permitted Aedes aegypti mosquitoes that were infected with P. gallinaceum to bite small White Leghorn chicks weighing between 75 and 100 grams each. The infectivity of the blood and various organs was tested by subinoculation into uninfected birds. During the first 36 hours none of the organs showed any evidence of infectivity except the muscle at the site of the bites. This first "negative" phase lasted for 36 hours. A very few evidences of infections were demonstrated in the blood and some of the organs between the thirty-sixth and the seventy-ninth hours, after which time the blood and the other tissues tested were consistently infected. The organs which were infected during the period between the thirty-sixth and seventy-ninth hours were the lungs, spleen and heart. No infections of the brain, bone marrow and pancreas were demonstrated during this period.

Similar tests were made to determine the infectivity of the blood and principal organs of chicks receiving intravenous inoculations of large numbers of sporozoites. It was demonstrated that infections were produced during the first 36 hours in the spleen, kidneys, liver, pancreas and muscles. The blood, however, did not become infective until the thirty-sixth hour, then it became negative until the eighty-second hour at which time it was again infected. All the tissues tested, with the exception of the brain and the bone marrow generally produced infection before the seventieth hour, and these two likewise became infected at this time.

When suspensions of sporozoites taken from 130 to 200 infected Aedes mosquitoes were inoculated intravenously into chicks, the presence of the

sporozoites in the blood was demonstrated for 5 to 20 minutes by subinoculation and up to 15 minutes microscopically, after which time the sporozoites disappeared. Infectivity of the blood did not reappear until 40 hours later.

Hegner and Eskridge (1938) attempted to determine which age of erythrocyte was attacked by malarial parasites. They observed infected red blood cells of P. relictum in canaries, pigeons and chicks, P. circumflexum in the canary and red-winged blackbird, and P. elongatum in canaries. Most of the parasites were believed to have entered the young red blood cells.

Hewitt (1939a) injected phenylhydrazine hydrochloride into 53 canaries before inoculating them with P. cathemerium parasites. Through the action of the drug, which was probably hemolytic, with a compensating hyperplasia of the hemopoietic system, there was a great increase in numbers of the young erythrocytes. After inoculation of both drug-treated and non-treated birds, it was found that a higher peak of infection was reached in the drug-treated birds. A possible explanation for this was that there were many more young cells for the parasites to infect, so there was less multiple infection of individual cells, and less parasite destruction during each asexual cycle, which resulted in a more rapid rise in parasite numbers and a higher parasite peak.

Boyd (1939) inoculated thirty canaries with P. cathemerium in order to study the reproduction activity of this parasite, and found that the rate of parasite destruction by the host was relatively low at the beginning of the infection, but rose rapidly as the disease progressed. However, the course of events during the early stages of a malarial infection occurred

at such a rapid rate, with the defense mechanism of the host reaching such a high degree of effectiveness that it was somewhat difficult to analyze or to predict with any degree of accuracy, all the factors which determined the course which any one infection would follow.

Wolfson (1940b) was able to cultivate successfully three species of Plasmodium in duck embryos. They included P. cathemerium, P. elongatum and P. lophurae. Not every embryo showed positive results, but some of each group of inoculations were positive. In some cases, if the blood was not positive for the parasites, subinoculation into adult ducks was shown to produce the infection in these birds.

Gingrich (1941a) performed some preliminary experiments on hyperimmunization by inoculating three canaries having latent infections of P. cathemerium with intravenous injections of 1 cc. of heat-killed parasites during a period of 21 days. Examinations of the blood were made before, during and after immunization. The fifth day after the last injection of killed parasites, 0.3 cc. of blood was taken from each of the three birds and inoculated into three uninfected canaries. Two of these became infected, with incubation periods of eight and ten days, but the third canary did not.

Huff (1941) attempted to find what the effect of passage of malarial parasites through mosquitoes would be. He passaged a strain of P. cathemerium through 24 canaries, by blood transfer, then infected mosquitoes with this by permitting them to bite the infected canaries. The mosquitoes (Culex pipiens) were permitted to bite canaries to give them the sporozoites from their bodies. Infections produced in all the birds mentioned parasites which seemed to be alike morphologically. From these

experiments it would appear that passage through the mosquito served no useful function. However, one of the strains tested by Huff had lost its ability to produce large numbers of gametocytes before passage through a mosquito. It regained this ability after it had been passed through mosquitoes.

Hewitt (1941) pointed out that in infections of canaries in which either P. relictum or P. cathemerium were used as the parasites, the blood, bone marrow, spleen and liver all showed concentrations of parasites at one time or another. Outside of the fact that the spleen and bone marrow usually contained fewer parasites than either the peripheral blood or the liver, there was found to be considerable variation in all the birds examined. Differences occurred among the individual birds in the distribution of the parasites as well as in the particular stages of the parasites present throughout either the patent period or the asexual cycle. During an infection, it was found that the stimulation of the hemopoietic system and the proliferation of phagocytic cells may change the general circulatory structure of some of the organs. If emboli and infarcts are produced, they may block off channels which in turn may result either in concentrating or in lessening the total number of certain stages of the parasites in the areas thus affected. The parasitic counts made from different parts of the spleen, liver or bone marrow were not always comparable, because variations in the structure of the blood vessels, or mechanical blockage resulting from emboli of infarction influenced the number of parasites found in the part examined. Hewitt showed that in vitro the malarial parasites clump around the mononuclear leucocytes and the endothelial cells, which may explain the concentration of parasitized cells found in blood vessels in some

parts of the spleen and liver. Chemical stimuli such as glucose, or high oxygen concentration may serve to attract the parasites to certain areas also.

Cannon (1941) pointed to the selective localisation of malarial parasites in such areas as the spleen, liver, and bone marrow as resulting from the anatomic and physiologic characteristics of these organs insofar as they possess blood sinuses in addition to capillaries in which the rate of blood flow is changed. In addition to localisation of blood at these sites, clumps of parasites may block the blood vessels in organs such as the brain, heart, and gastric mucosa, giving rise to lesions.

Coulston and Huff (1947) inoculated different hosts with strains of P. relictum in order to observe the infectivity in the various hosts. Strain 1P of P. relictum from pigeons was found to be transmissible by blood inoculations to both pigeons and canaries, but did not infect mosquitoes. However, strain 1Pl of canaries was found to be transmissible by blood inoculations to pigeons and canaries as well as to Culex pipiens mosquitoes. Sporozoites of strains of 1Pl as well as of 1Pl-1 produced cryptozoites in the skin and only subpatent infections in the blood of the pigeons. Infected blood of strain 1Pl-1 from canaries, inoculated intravenously into pigeons, produced initial but transient parasitemia in the pigeons, but these infections became subpatent and persisted for periods of as long as 61 days.

Weight of the host and its relation to malaria

Terzian (1941b) observed that young Rhode Island Red chicks, Barred Plymouth Rocks, White Leghorns and White Rocks were equally susceptible to

infections with P. lophurae as long as the factors of weight and parasite dosage were constant. Age of the chick as a factor in determining the extent of an infection seemed to be important only insofar as it was related to the weight of the bird. Data indicated that increased resistance to the parasite results from an increase in weight. However, the degree of increased weight of the bird was related geometrically to the parasite numbers rather than arithmetically, since the increase in resistance to the parasite was entirely out of proportion to the relative weight of the bird. This was demonstrated by inoculating chicks weighing 50 grams each with parasite dosages of 50×10^6, and chicks weighing 100 grams each with a dosage of 50×10^6 parasites. On the first day following inoculation the average number of parasites per 10,000 red blood cells for the 50-gram chicks was 120, and only 80 for the 100-gram chicks. The average on the fifth day for the 50-gram chicks was 4,900, and only 640 for the 100-gram chicks. By the tenth day all but one of the 50-gram chicks were dead, whereas all the 100-gram chicks were alive and their blood seemed to be cleared of the parasite.

Temperature responses to malaria infections in avian hosts

In his experiments with mature ducks, Hewitt (1942) observed that during the first two or three days after inoculation with P. lophurae the temperature fluctuations of parasitized birds and the parasitized controls showed very little difference except for the few birds which reacted with what seemed to be a temperature response on the day following inoculation of the parasites into the blood stream. On approximately the third day, however, as the parasite numbers began to show a rapid increase, the temperatures of the infected birds also showed a rise, with sometimes as

much as 6° F. within a 24-hour period. The peak of parasitemia was generally reached during the fifth or sixth day after inoculation, and as this occurred the temperature usually dropped somewhat abruptly, regardless of whether the number of parasites continued to rise and the bird died, or whether the parasite number dropped rapidly and the bird recovered. Hewitt found the rectal temperatures for unparasitized ducks he examined averaged approximately 106.2° F. for birds kept at a constant environmental temperature, and 107.8° F. for birds kept in an outdoor cage. During the malarial infection, however, the temperatures of the birds reached as high as 111° F. Following high peaks of parasitemia, or just preceding death, subnormal temperatures were frequently observed.

Huff (1939) using two strains of P. cathemerium and P. relictum parasites attempted to show changes in temperature of canaries inoculated with these parasites as compared with uninoculated control birds. No temperature rises were found in the canaries accompanying the periods of greatest segmentation of asexual forms. Neither were there any significant differences observed in the temperatures of the pectoral muscles of infected and uninfected birds. The range of temperatures of individual birds varied as much as 6° C. over a 24-hour period. Average daytime temperature of birds was about one degree higher than that for the night temperature.

Effects of nutrition on the course of malaria

Brooke (1945) pointed out that the occurrence of malnutrition and malaria among the human population is a common phenomenon in many parts of the world. Parts of the south in the United States where malaria

flourishes most highly, correspond with some of the most poorly nourished regions of the country. He referred to the studies of Cristophers (1911); Gill (1928); Martini (1928); and Dickson (1935) which showed that epidemics of malaria frequently accompany or follow periods of famine, or periods of economic stress during which time the inhabitants of an area are under-nourished or malnourished. Likewise, he indicated that the investigations of these men showed that under such conditions malaria is generally reported as being more intense, fatal, and more prolonged than malaria existing among comparatively well-nourished populations. Brooke (1945) further pointed out the work of Bentley (1911) which indicated that economic stress enables low grade infections to persist, and favors the reappearance or relapse of the disease. On the other hand, Celli (1925) believed that the major cycles of malaria down through the centuries were due to an inherent periodicity of the disease. Hackett (1937) wrote of the close relationship existing between economic stress and malaria. He maintained that during periods of great social upheaval and poor economic conditions, malaria springs up.

Although a great deal has been written as well as said concerning the relationship existing between malaria and poor nutrition, extensive experiments have been carried out only since about 1940. Passmore and Sommerville (1940) reported that they found no significant difference between the course and severity of the malarial infections in Macacus radiatus monkeys that were given a well-balanced diet and the ones placed on a poorly balanced diet. The well-balanced diet resembled a good, human, lactovegetarian diet such as that used by some of the people of

northern India, whereas the poorly balanced diet was similar to the type eaten by the poorer rice-eaters of India. Two experiments were set up, using P. cynomolgi for one and P. knowlesi for the other. In both experiments the average number of parasites was very slightly lower in the monkeys given the ill-balanced diet, while the lengths of the pre-patent and the patent periods were practically the same in the two diet groups. Observations were made on the relapses following splenectomy of two monkeys on each of the diets but no significant differences were apparent.

Trager (1943b) presented his results demonstrating that a biotin deficiency greatly affected the susceptibility of avian hosts to primary attacks of malaria. He used ducks and chickens as the host birds, and P. lophurae, P. gallinaceum and P. cathemerium as the infective agents. When compared with the controls, it was found that the birds which had been rendered biotin deficient by an egg-white diet generally had parasite peaks which were higher, parasite numbers which persisted at a high level for several days longer, and infections that resulted in a greater number of deaths than the control birds. It was observed, however, that negative results were obtained when a diet that was deficient in pantothenic acid was used, as the birds on this diet did not have any heavier infections than the control birds on the regular diet.

Brooke (1945) used canaries, pigeons, and ducks in his experimental work to show the effects of diet. Canaries were inoculated with P. relictum and P. cathemerium, pigeons with P. relictum, and ducks with P. lophurae. Most of the birds which were given the poor diets suffered more severe primary attacks of malaria than did the controls. Generally,

these primary attacks were characterized by having a greater number of parasites present than did the controls, they suffered higher parasite peaks, longer patent periods, and showed more severe symptoms. Excluding the few instances in which death apparently resulted directly from the effect of the deficient diets, the mortality rate was greater among the birds on the poor diets than among those on the stock diet. In the experiment using birds which had latent malarial infections, it was demonstrated that deficient diets could adversely affect the degree of immunity acquired from primary attacks. This was demonstrated when it was observed that parasite relapses occurred only among the birds which had been given poor diets, and that the degree of immunity to superinfection was greatly reduced or entirely gone in a number of the nutritionally deficient birds.

With the exception of a diet which was materially reduced in quantity, as well as deficient in vitamin B_1, Brooke (1945) used experimental diets that were high in carbohydrates and low in practically all other nutritional values when compared with the control diets. These deficient diets were somewhat comparable to the diet consumed by many people of malarious regions of the southern United States, as well as in other countries. The birds on the test diets probably suffered from undernourishment as well as from malnutrition, since their appetites were usually affected in an adverse manner.

In the experiments using birds that had latent infections with malaria, Brooke (1945) found that parasites appeared in the peripheral blood only of birds that were given the experimental diets. A diet which was deficient only in vitamin B_1, however, was not found to provoke any malarial relapses in pigeons which had latent infections, no matter how

severe the specific deficiency symptoms became, and furthermore it did not seem to lower their resistance to superinfection. Immunity to superinfection was greatly reduced, or entirely broken, in more than one-third of the birds having latent infections, and which were given the experimental diets, and in one bird on the control diet. One pigeon which was superinfected while on a rice diet regained its immunity after being placed on a stock diet for a period of 11 days.

Seeler and Ott (1945) observed that P. lophurae infections were more severe in chicks which were given protein-deficient diets than in chicks which were given diets containing adequate amounts of protein. The parasite counts reached much higher levels in the deficient birds than in controls on a high protein diet, and the mortality due to the disease was greater in the deficient birds. In addition, the protein deficient chicks were not able to clear the parasites from their blood streams as readily as were the birds fed a diet containing ample amounts of protein.

Norris and Ringrose (1930) described a "pellagrous-like syndrome" observed in chicks, with eyelids that were granular, sticky, and so contracted that vision was restricted, when either dried raw egg white or purified casein was used as the major part of the protein in the diet. The addition of two and five tenths percent of autoclaved yeast to the purified-casein diet improved growth of the birds and prevented granulation of the eyelids.

The work of Kline, Keenan, Elvehjem and Hart (1932) demonstrated that the severity of dermatosis in chicks fed a purified casein diet was greatly increased by subjecting the diet to prolonged heating at high temperatures in a dry atmosphere.

Lease and Parsens (1934) reported that the factor which prevented the dermatosis in chicks fed a heat-treated, purified casein diet was not identical with the one which prevented the dermatosis in chicks fed a raw egg white diet. Some of the investigations which seemed to confirm this included the work of Ringrose, Norris and Heuser (1936a, 1936b); Jukes (1939a, 1939b); and Woolley, Waisman and Elvehjem (1939a, 1939b). They demonstrated that the factor which prevented the dermatosis in chicks fed a heat-treated purified casein diet was pantothenic acid. Eakin, McKinley and Williams (1940); Eakin, Snell and Williams (1940); and Hegsted, Oleson, Mills, Elvehjem and Hart (1940) indicated that the factor which prevented the dermatosis in chicks fed a raw egg white diet was biotin.

In the experimental work of Bauernfiend, Norris, and Heuser (1942) it was found that Single Comb White Leghorn chicks required from 500 to 550 micrograms of pantothenic acid per 100 grams of diet for the prevention of dermatosis, and approximately 600 micrograms per 100 grams for maximum growth. Rhode Island Red chicks were found to be less susceptible to pantothenic acid deficiency than Single Comb White Leghorns, and their requirement was approximately 75 microns less per 100 grams of the diet.

Corradetti (1940) believed that italchina delayed the appearance of exoerythrocytic schizonts of P. gallinaceum in chickens, and that the action was directed against the erythrocytic parasites from which he believed some of the exoerythrocytic schizonts are produced.

Trager (1943a) observed that the presence of pantothenate favored the survival in vitro of P. lophurae. However, Trager (1943b) also found that infected chicks and ducklings which were given a pantothenic acid

deficient ration experienced neither heavier nor lighter infections than those fed the complete ration. However, a ration that was deficient in biotin so lowered the resistance of the birds given this diet that they suffered a higher parasitemia than birds given the control diet, and more deaths occurred among these birds due to the higher parasitemia.

The work of Bracket, Waletzky and Baker (1946) pointed out that in a study made of blood-induced infections of P. gallinaceum in chicks, significantly higher parasitemia occurred in birds which were given the pantothenate-supplemented ration than in those on the deficient diet. This was contrary to the results obtained by a number of other workers, such as Seeler, Ott and Gundel (1944), who obtained higher peak parasite counts in P. lophurae infections in chicks that had been placed on a diet deficient in biotin than in chicks receiving an identical ration but containing adequate amounts of the vitamin.

Becker, Brodine and Marousek (1949) found that eyelid lesions appeared among chicks of one group which had been placed on the special G-ration. They appeared only in the birds which had been infected with P. lophurae and fed on the unsupplemented G-ration. Although other rations were tested, they failed to produce chicks suffering from the eye lesions. In its most severe form, the eyelid affliction involved the encrusting and eroding away of the lower eyelid, followed by a healing process during which time there was a regeneration or a replacement of the lost eyelid. In milder cases, a prominent notch was produced in the margin of the lower eyelid. It appeared that the development of the lesion did not depend on the absolute intensity of the parasitemia, but to the attainment of a certain density of parasitemia within a definite period

of time after inoculation. Indications showed that the disorder resulted from an acute dietary deficiency precipitated by competition for biotin and for pantothenic acid between the host and the vigorous parasite population reproducing at its maximum rate. Apparently, the affliction was forestalled when small amounts of either biotin or pantothenic acid were added to the ration.

The research of Couch, Cravens, Elvehjem and Halpin (1949) pointed out the appearance of notched upper eyelids which they observed in embryos and chicks from eggs which had been inoculated with five units of insulin 120 hours after incubation had started. It is presumed that the insulin treatment produced biotin deficiency, which in turn was demonstrated in a number of deformities, among them being the notched eyelids.

Trager (1948), working with White Pekin ducks which were from six to seven months of age and which had been inoculated with P. lophurae, observed that in nine of the twelve ducks whose ovaries were active at the time of the inoculation, the parasites underwent little or no multiplication. All eight of the females whose ovaries were inactive at the time of the inoculation developed heavy infections. This was also true of the ten males that were tested. The average free-biotin and bound-active lipoid material of the plasma prior to inoculation were the highest in the egg-laying females, lowest in the males, and intermediate in the females that had inactive ovaries. It would appear, from these experiments, that the presence or the absence of available biotin is tied up with the development or non-development of the parasites within the body of the host.

MATERIALS AND METHODS OF PROCEDURE

Strain of *Plasmodium lophurae*

The strain of *P. lophurae*, avian malarial parasite, used in the present experimental investigations was one which Dr. E. R. Becker received in 1947 from an infected duck supplied by Dr. William Trager of the Rockefeller Institute of Princeton, New Jersey. This strain had been maintained by Dr. Becker by transmitting it serially through young White Pekin ducks by means of intravenous injections of moderately heavy doses of parasitized red blood cells at intervals of four, five or six days.

Chicken Hosts

The chicken hosts used in the experiments consisted of six groups of Rhode Island Reds which were brought to the laboratory at various periods of time directly from the hatchery and placed on a commercial chick starter obtained locally and found to be satisfactory for feeding purposes. The mash did not contain supplements of antibiotics or other therapeutic or prophylactic drugs. Size of the groups varied from 10 to 18 chicks. Each group was kept on the regular starting rations for at least two days before being transferred to any special rations. The special rations had been weighed out and hand mixed in the laboratory. A list of the contents of the various special feeding rations is included later.

Several days prior to their inoculation with an infective dose of

P. lophurae, a metal plate with an identification number was attached to a wing of each chick so that it could be considered individually in computing results throughout the course of the investigation.

Duck Hosts

The ten ducks used in the research were the White Pekin type, which were brought to the laboratory when they were one day old, and placed on the same commercial starter as that given the chicks. This was the only ration which they received during the entire course of the experiments.

Turkey Hosts

The two turkeys used for experimental purposes were the bronze-breasted type. They had been obtained from the hatchery when one day old, and raised on the same commercial starter given the chicks and ducks.

Source of the Inoculation Material

Inoculum for chicks

The source of the parasitized red blood cells used for inoculating the chicks was blood taken from young passage chicks in which the number of red blood cells parasitized by P. lophurae had reached a level of infection varying between 70 and 80 percent. This level generally occurred about the fourth or fifth day after inoculation. Previously these passage chicks had been inoculated with blood from ducks in which P. lophurae was present.

Inoculum for ducks

The ten ducks were inoculated with a saline emulsion made from the brain tissues of two turkeys whose blood smears had indicated that there were no malarial-infected red blood cells present. The capillaries of their brains, however, showed the presence of a great number of exoerythrocytic forms (phaneroscites) of P. lophurae.

Inoculum for turkeys

The two turkeys whose tissues were examined had been infected with blood taken from a young passage duck whose red blood cell count showed that approximately 70 percent of these cells were infected.

Method of Inoculation

Inoculation of chicks

Blood was taken from the jugular vein of a passage chick in which it had been demonstrated that between 70 and 80 percent of the red blood cells were parasitized with P. lophurae. The blood was placed in a tube with an anticoagulant consisting of 1/2 cc. of 0.8 percent sodium citrate solution in the bottom of a 15 cc. glass tube. After being mixed with the anticoagulant, about 5 cc. of the citrated blood was placed in a screw cap test tube and centrifuged at about 2,000 r.p.m. for a period of five minutes, then the supernatant plasma was removed. Ten cc. of physiological salt solution was added to the residue of cells and the mixture was shaken and mixed thoroughly, then centrifuged again for five minutes. After three or four such washings the red blood cells were suspended in physiological

salt solution to obtain the proper concentration of inoculum. Computation for obtaining the desired concentration was based on the parasite counts made on dried blood smears which had been stained with Giemsa stain, just prior to the drawing of the blood from the jugular vein of the chick donor. Infective dose was 0.8×10^8 infected red blood cells per 100 grams of body weight of chick. The solution of infected red blood cells was injected intravenously into the left wing of each chick.

Inoculation of ducks

For inoculating the ducks, the entire brain was removed from each of two turkeys whose brain capillaries had shown a very high concentration of exoerythrocytic schizonts (phanerozoites), and diluted 1:20 with normal saline. Approximately 0.9 cc. of the emulsion was injected into each of the five-day-old ducks without regard to their weight. As in the chicks, the inoculations were made intravenously in the wings.

Inoculation of turkeys

The turkeys were inoculated in the same manner as the chicks, but the source of the infected red blood cells was ducks whose blood showed a high rate of infectivity.

Age and Weight of Host Birds on Day of Inoculation

Chicken hosts

Inoculation date for the chicks used in the experiments varied from the twelfth to the fourteenth day of life. However, the average weight

for each of the groups tested, and for the individual chicks, showed a wide variation, with the average weight for some of the 12-day-old chicks greater than that for some of the 14-day-old chicks. The lowest recorded was for one group of 12-day-old chicks whose average weight was 67 grams, whereas the highest was for one group of 13-day-old chicks with an average weight of 92.8 grams.

Duck hosts

The ten ducks serving as hosts to the exoerythrocytic stages of P. lophurae were inoculated when they were five days old. The average weight of the ducks on the day of their inoculation was 63.7 grams, although individual weights varied considerably.

Turkey hosts

The two turkeys used in the experimental work were inoculated with infected red blood cells from a passage duck in the same manner as the chicks. One of the turkeys was inoculated when it was five days old and the other when it was six days old. Weight records were not kept.

Observations of Infected Red Blood Cells, Temperature and Weight of Chicks

For observations of the number of infected red blood cells, blood smears were made and stained with Giemsa stain at approximately the same hour each morning starting with the first day following the inoculation and continuing until the chicks died, or until no more infected red blood cells could be demonstrated in their blood. The procedure followed for

counting the cells is indicated later.

At the time the chicks were given their identification numbers, each one was weighed. A daily record made at approximately the same time each day was kept of the losses or gains in weight of each chick from the day that it was first identified by number until it died following inoculation with P. lophurae, or was sacrificed, or until its blood no longer contained malarial parasites.

Daily rectal temperatures of chicks were made at about the same hour each morning, beginning a few days prior to the inoculation date and continuing for the same period of time as is indicated for the weight experiments.

Observations were made of the patterns followed for each of these factors of chicks within the same group and same diet, as well as observations and comparisons made between the groups given different diets.

Observations Made on Ducks Inoculated with Phanerozoite Stages of P. lophurae

After the ducks were inoculated with exoerythrocytic stages of P. lophurae from turkeys who had died, blood smears from leg veins were examined daily for the presence of the malarial parasites. As was true of the chicks, weight records were also kept for the ducks, until they became so unwieldy that it was not deemed feasible to weigh them any more. As each duck was sacrificed, sections, smears and imprints were made of tissues from the various organs as listed for the chicks. Microscopic examinations were similar to those listed for the chicks.

Observations of Turkeys Inoculated with Blood Stages of *P. lophurae*

The only observations made on the turkeys, one of which apparently died of the exoerythrocytic stages of *P. lophurae*, were microscopic examinations of imprints, smears and sections of the same organs as the ones listed for the chicks. Staining was similar to that for the chicks.

Eye Lesions in Chicks

The chicks fed on all the diets were observed daily for the possible appearance of eyelid lesions. This phenomenon had been observed by Becker, Brodine and Marousek (1949) among chicks which had been fed the same G-ration as given some of the experimental chicks in the present research.

Exoerythrocytic Stages in Chicks and Ducks

As one of the main considerations of the present research was the attempt to find exoerythrocytic stages, with emphasis on the phanerozoites, the organs and tissues of birds which had died or had been sacrificed were examined microscopically for the presence of these forms. Smears, imprints and slides were made of liver, spleen, lung, adrenal, kidney, heart, brain, pancreas, stomach, bone marrow and eyelids. There was a total of 50 chicks, 10 ducks and two turkeys of varying ages, weights, and degrees of infection by malarial parasites. Slides which contained imprints or smears of various organs were stained with Giemsa, but the slides of the sections were stained with toluidine blue and phloxine. Examinations were with the

oil immersion lens.

Procedure Followed for Counting the Parasitized Erythrocytes in Giemsa-Stained Blood Smears

1. If 0, 1, 2, 3, 4, or 5 parasitized red blood cells were found in the first 100 red blood cells counted, the counting was continued until 3,000 red blood cells were counted, or until 50 parasitized red blood cells were counted, using which ever number was reached first.

2. If 6 through 10 parasitized cells were found, 500 red blood cells were counted.

3. If 11 through 13 parasitized cells were found, 400 red blood cells were counted.

4. If 14 through 17 parasitized cells were found, 300 red blood cells were counted.

5. If 18 or more parasitized cells were found, 200 red blood cells were counted.

The percent of infected red blood cells was computed directly by dividing the number of parasitized red cells by the number of hundred red blood cells counted.

Examples to illustrate the method of computing the percent of infected red blood cells:

Example 1.

If 48 parasitized red blood cells were found in 3,000 red blood cells counted, the percent of parasitized cells was 48 divided by 30, or 1.6%.

Example 2.

If 50 parasitized red blood cells were found in 2,250 red blood cells counted, the percent of parasitized cells was 50 divided by 22.5, or 22.22%.

Example 3.

If 64 parasitized red blood cells were found in 200 red blood cells counted, the percent of parasitized cells was 64 divided by 2, or 32%.

For counting the cells, an area of the Giemsa-stained blood smear was selected about one-third of an inch from the end of the smear where the red blood cells were evenly distributed over the slide and where they numbered approximately 100 in each field under the oil immersion lens. The first 100 red cells observed was frequently an index to the number of cells that had to be counted to give a parasite estimation, with a probable error of 10 percent. However, this did not allow for non-random factors in the distribution of the parasitized cells, and did not hold true for counts of less than 150 parasitized cells per 10,000 red blood cells.

Regular Chick Starting Ration

Analysis of contents

Crude protein, at least	-	20.00 %
Crude fat, at least	-	3.00
Crude fiber, at least	-	7.50
Minerals, at least	-	4.25

Ingredients of the contents

Included in the ingredients were ground yellow corn, ground oats, wheat middlings, wheat bran, soybean oil meal, meat scraps, dehydrated alfalfa meal, dried buttermilk, vitamin A and D feeding oil, D-activated animal sterol, limestone and salt. In addition to these ingredients, 2.50 percent of Hess Feed Builder for poultry was included. This contained the following materials: bone black, steamed bone meal, defluorinated phosphate, animal protein factor supplement which was the source of vitamin B_{12}, rock phosphate, iron sulphate, iron oxide, manganese sulphate, dried grains and skim milk fermentation solubles (which were the source of riboflavin), copper sulphate and potassium iodide.

G-Ration

Analysis of contents by weight

Yellow corn meal	-	32.00 parts
Grey wheat shorts	-	25.00
Ground hulled oats	-	15.00
Wheat bran	-	10.00
Alfalfa meal	-	5.00
Dried powdered buttermilk	-	5.00
Tankage	-	5.00
Limestone	-	2.00
Salt	-	0.75
Cod liver oil concentrate	-	0.25

According to the statement by the manufacturer, the cod liver oil

concentrate contained 3000 U.S.P. units of vitamin A and 400 A.O.A.C. chick units of vitamin D per gram.

Q-Ration

Analysis of contents by weight

The Q-feeding ration was made up of equal parts of G-ration and the following mixture (B):

Yellow corn meal	-	58 parts
Wheat middlings	-	25
Washed casein	-	12
Calcium carbonate	-	1
Bone meal	-	2
Salt	-	1
Vitamins		
Thiamine	-	1 mg./kg. of B mixture
Riboflavin	-	1 mg./kg. of B mixture
Vitamin K	-	10 mg./kg. of B mixture

This ration was heated to 120° C. for a period of 24 hours, using dry heat to destroy vitamin B_2 and vitamin B_4. This is based on the work of Hegsted et al. (1940).

R-Ration

Analysis of contents

This ration was identical with the Q-ration except that 25 parts of ground hulled oats were used in place of the wheat middlings.

S-Ration

Analysis of contents

This ration was identical with the Q-ration except that tankage was used in place of the casein.

Staining Technique for Tissue Studies

Much of the investigation was centered around the examination of various tissues of the body for the presence of exoerythrocytic stages of P. lophurae and for organs in which phagocytosis of malarial pigment and parasitized red blood cells occurred. If a chick was found dead in its cage, or if it was sacrificed, various organs were removed from its body and placed in formol-saline solution or Bouin's fixative for a period of at least 24 hours. Later they were dehydrated, cleared and infiltrated with paraffin. These were made into sections measuring five microns in thickness, placed on slides and stained according to the technique outlined by Tomlinson and Grocott(1944) using toluidine blue, phloxine and orange G for staining purposes. Tissue nuclei were stained blue to purple with the cytoplasm pale rose-red. Supporting stroma of the tissue stained a pale-orange red. This seemed to be the only place where the orange G was at all noticeable in the cells. The erythrocytes stained brilliant red to orange-red depending upon the fixative and whether the erythrocytes were congested in the tissue or were present in smaller numbers. The malarial parasites appeared as pale blue cytoplasmic structures with sharp margins, observed within the erythrocytes. The nuclear chromatin varied in color

from a deep purple in the older trophozoites to a lavender-rose in the young trophozoites.

For making imprints, the organs were cut across and touched lightly to a slide, fixed in methyl alcohol, dried, then stained with Giemsa stain.

For making smears of the organs, a small piece of tissue was placed on a slide, then macerated with a second slide. After removing the surplus bulk of the tissue from the slide, the macerated tissue was fixed in methyl alcohol and stained with Giemsa stain.

Organs from 50 of the chicks that had died or were sacrificed were made into slides. These included sections, smears and imprints of organs from 50 chickens, ten ducks and two turkeys.

Organs examined were the kidneys, liver, spleen, lungs, brain, adrenals, pancreas, bone marrow, eyelids, intestine, heart, gizzard, rectum and gonads. Not all the tissues listed were made into slides for each bird. As the investigation progressed, it did not seem feasible nor of value to include examination of all the tissues.

DISCUSSION AND RESULTS

Chickens

Parasitemia, weight and temperature changes

The presentation of the results is included in the Appendix in the form of tables showing comparisons of the parasitemia, weight and temperature changes of the chicks within the groups fed the same rations as well as those given different rations. For the tables showing the daily changes in the number of infected red blood cells present on the various days, records are indicated for the first through the tenth day following inoculation of chicks with 0.8×10^8 parasitized red cells with P. lophurae per 100 grams of chick body weight. Although in some instances the blood was not yet cleared of parasites by the tenth day, the general tendency was a downward trend in the number of parasites that could be demonstrated in Giemsa-stained blood smears. Responses among chicks of the same group, as well as those among different groups, varied considerably.

The chicks in the 1 A series (Tables 1, 10 and 20) were placed on the G-ration when five days old, and were inoculated with P. lophurae at the age of 12 days. On the day following their inoculation, the group mean for parasitized red blood cells was 1.31 percent. The lowest percentage recorded on the day following inoculation in this group was for chick No. 1461 with 0.93 percent of the cells infected, and the highest was for chick No. 1459 with 2.11 percent. However, the chick with the lowest

parasitemia the first day had one of the highest recorded parasitemias on its peak day, but this appeared at a later date than did the highest parasitemia of most of the chicks for the 1A series. Chick No. 1459 reached its peak of parasitemia earlier than did most of the other chicks in the group, but it became very ill, and its weight dropped rapidly following the high point in its parasitemia. For all the chicks in this group, it was observed that, in general, on the day prior to the peak of the parasitemia, the temperature tended to be higher than on other days of the parasitemia. With the parasitemia reaching its peak, there was a fall in the temperature of the chicks. Following the peak day of the parasitemia, the weight of the chicks in the 1 A series tended to fall also. However, within a few days the weight began to increase as the number of parasites in the blood became less.

A deviation from the general pattern was followed by chick No. 1462. On the sixth day following inoculation with P. lophurae it attained a parasitemia of 37.5 percent. On the following day there was a decrease in the number of blood cells which were found to be infected to the extent of 17.0 percent. However, on the eighth day the parasitemia reached a new high of 45.5 percent, followed the next day with 23 percent. On the tenth day the chick was dead.

The chicks in the 1 B series, Tables 2, 11 and 21, were given the regular chick ration for the entire period of the experiment. Like the chicks of the 1 A series, they were inoculated when 12 days old. On the first day following inoculation with P. lophurae, the mean of the parasitemia for the entire 1 B group was slightly higher than the mean of the parasitemia for the 1 A chicks. For the 1 B chicks the greatest

parasitemia occurred on either the sixth or the seventh day. Chick No. 1464 with a parasitemia of only 0.83 percent following the day after inoculation developed one of the lowest parasitemias of the group with its highest peak on the seventh day. Chick No. 1466, which had the highest number of parasitized red blood cells present on the day following the inoculation, exhibited the same pattern as chick No. 1462. It attained a peak of 34.5 percent parasitemia on the sixth day following inoculation, then the seventh day fell to 20.0 percent, but on the eighth day was up to 38.0 percent. Although on the day these chicks were inoculated their mean weight was ten grams below that for the 1 A series, their mean weight on the tenth day was just three and one-half grams under that for the 1 A series. It might be pointed out that the first time the chicks of the 1 A and the 1 B series were weighed the chicks in the 1 A group averaged about 4 grams heavier than those in the 1 B series. Thus the chicks in the 1 B series had a weight handicap to overcome, and may not have been as strong as the ones in the 1 A group in the beginning. In every instance the temperature of the chicks was the highest on the day before the peak day of the parasitemia.

The chicks of the 1 C series (Tables 12 and 22) cannot be compared in number of infected red blood cells with the other groups mentioned, as they were not inoculated with the malarial parasites. They were given regular rations throughout the investigation. On the day that the other chicks were inoculated, the mean weight of this group was 69 grams, which was slightly higher than the mean of 67 grams for the 1 B group, but lower than that of the 1 A series, which was 77 grams. Growth proceeded in this group at a regular and more or less even rate until the tenth day when the

weight reached 125 grams, as compared with 107.5 for 1 A series and 104 for the 1 B series. The temperature showed very little change from day to day. The lowest temperature on any one day was 107.0° F., with the high at 107.2° F. This group which was non-inoculated and which received a normal chick diet showed less change in temperature and a more even growth than did any group tested.

Chicks of the 2 A series (Tables 3, 13 and 23) were placed on the G-ration when only three days old. Only two of the ten chicks started on the G-ration were alive on the tenth day of the experiment. In both cases these two still showed parasites to be present in their blood. Chicks of this group were inoculated with P. lophurae when 12 days of age, and at that time, only four of the original ten were alive. The mean weight for the group on the inoculation date was 74.4 grams, which was the lowest for any of the test groups of chicks used. Parasitemia the day following inoculation varied from a low of 2.02 percent to 4.17 percent. By the fourth day following inoculation all of the chicks showed a percentage of infected blood cells greater than 38 percent. The chick showing the lowest parasitemia (chick No. 1624), with only 38.67 percent of its red blood cells parasitized, died the following day. Chick No. 1619 attained a parasitemia of more than 54 percent on the fourth day, and then for three days held a parasitemia of more than 50 percent. Another of the group, chick No. 1626, also had more than 50 percent of its red blood cells infected with P. lophurae for a period of three days, but it continued to live and apparently recovered. There was a fall in the weight of the chicks on the day when the peak parasitemia was reached in all of the chicks of the 2 A series. Although there were only two chicks alive by the end of

ten days, these two had apparently recovered. Their weight averaged 121 grams. The highest temperature readings occurred on the day preceding the peak parasitemia, with a fall in the temperature observed on the day of the peak parasitemia. Chick No. 1626 which showed a parasitemia greater than 50 percent for a period of three consecutive days, and then apparently recovered, had two high points in its temperature record. These periods occurred on the third and the fifth days following inoculation. In each case they occurred on the day preceding the two peak points of the parasitemia.

The chicks of the 3 A series (Tables 4, 14 and 24) were taken off the regular chick rations and placed on G-rations on the fifth day of life. Of the 12 chicks used for the 3 A series, eight were still alive after the tenth day of the infection, despite the very high number of red blood cells which had been parasitized with P. lophurae. The lowest parasitemia following the day of the inoculation was 3.03 percent; the highest more than 6.08 percent. All the chicks in this group showed the highest parasitemia on the fifth day, with the exception of one chick, No. 1690, which showed the highest parasitemia on the fourth day. Even so, the number of infected red blood cells on the fifth day was still high (68.29 percent); but after a parasitemia of such magnitude for a period of two days, this chick died. The trend of the temperature readings corresponded to that of the other groups. Generally on the day before the peak parasitemia was reached, there was a rise in the temperature and a drop on the day of the peak parasitemia. Although the mean weight for this group was very high (92.8 grams) on the day the chicks were inoculated, all the chicks with the exception of two showed a drop in weight as the blood parasites increased

in number. The two exceptions to this trend were chicks No. 1689 and No. 1691. Chick No. 1689 showed a rapid rise in parasitemia, and on the fourth and fifth day the percent of infected red blood cells was greater than 48 percent. The following day the chick died. None of the chicks in the 3 A series showed a second rise in parasitemia after the peak had been reached.

The ration designated as Q-ration was given to the 15 chicks of the 4 A series (Tables 5, 15 and 25), commencing at five days of age. When they had reached the age of 14 days, they were inoculated with P. lophurae. Mean weight for the group on the day of inoculation was 80.27 grams. Even though the inoculation age for this group was older than for any of the other groups, their mean weight was at about the mid-point for all the groups. The lowest mean weight for any group was 67 grams for the 1 A series chicks, and the highest was 92.8 grams for the 3 A series. All the chicks of the 4 A series showed a parasitemia as great or greater than 28 percent on the fifth day. Four of the chicks continued to show an increase in the number of parasitized red blood cells on the sixth day.. Chick No. 1852, which showed a parasitemia of 28 percent on the fifth day following inoculation with P. lophurae, showed a decrease in the number of infected red blood cells the sixth day. However, on the following, or seventh day, a new parasitemia peak of 35 percent was reached. On the day prior to the first high parasitemia for this chick the temperature was 107.6° F., the highest recorded for chick No. 1852. However, following that day until the death of the chick on the eleventh day following inoculation, the temperature was lower each day, until on the tenth day it was only 102° F. The weight of this chick was also lower

than that of the rest of the group throughout the investigation. The weight of chick No. 1852 was the lowest of the 4 A chicks on the day they were first weighed. This relationship was not changed throughout the entire investigation, except on the day following inoculation, at which time two of the chicks weighed one gram less than did chick No. 1852.

Chicks of the 5 A series (Tables 6, 16 and 26) were placed on the diet known as R-ration when they were five days old, then inoculated with P. lophurae when they were 12 days old. Forty percent of these chicks reached a high peak of parasitemia varying from only 18 percent to more than 33 percent on the fifth or sixth day of the infection, followed by a drop in number of parasitized cells the next day, with a rise on the second day. One other chick of this group followed this same general pattern, but the first high parasitemia occurred on the eighth day and the second peak came on the tenth. All of the chicks of this series were still alive on the tenth day following the inoculation. On the day of their inoculation, these chicks averaged in weight second only to the chicks of the 3 A series. Respective means on inoculation dates were 92.8 grams for 3 A series chicks but 86.5 grams for the 5 A chicks. On the tenth day of the infection, the mean average weight for chicks of the 3 A series was 122.63 grams, and for the chicks of the 5 A series it was 116 grams. There was, however, a great difference in the number of chicks that survived until the tenth day. By the time the tenth day was reached, 33 percent of the chicks from the 3 A series had died, whereas 100 percent of those that had been placed in the 5 A series at the first of the experimental work were still alive and appeared to show no ill effects of the parasitemia. A comparison of the highest day of the parasitemia showed that

the chicks of the 3 A series attained high parasitemias by the third day, and that a continued rise occurred for two more days in most cases. In the chicks of the 5 A series, parasitemias in general did not show as high a level as in the 3 A series, but there was more of a tendency to attain a relatively high parasitemia, which was followed by a day of lesser parasitemia, then a higher one. The daily weight changes in the 5 A chicks showed less tendency to fall than in most of the groups. Individual chicks showed some few slight falls, but they were the exceptions rather than the rule. There seemed to be very little correlation in the 5 A chicks between height of parasitemia and the rise and consequent fall of the temperature. Fifty percent of the chicks showed their highest temperature to fall on the fifth day of the infection, with the rest occurring on the sixth, seventh or eighth day.

The ten chicks of the 5 B series (Tables 7, 17 and 27) were placed on the Q-ration the fifth day of life, then inoculated with P. lophurae when they were 12 days old. The mean weight of this group on the day they were inoculated was 82.7 grams. This was 3.8 grams less than the mean for the 5 A chicks which were inoculated at the same time. However, on the tenth day following inoculation the mean weight for the 5 A group was 116 grams, while that of the 5 B chicks was only 95.67 grams. All except one of the 5 B chicks were alive on the tenth day following inoculation with P. lophurae. Two-thirds of these chicks followed the same general pattern of two peak parasitemias as that which occurred in the 5 A chicks. A relatively high parasitemia was reached which was followed by one or more days during which time the blood parasites occurred in lesser numbers, then a second peak parasitemia was reached. In some instances the second peak

was greater than the first. There appeared to be no single day which favored a high mean parasitemia, but on the eighth day of the infection the mean parasitemia for the group was slightly higher than that for any other day. However, the range for the mean parasitemia for the sixth, seventh, eighth, ninth and tenth days following inoculation was only between 22.56 percent and 24.75 percent. The days on which the peak parasitemia occurred were not in accordance with the results obtained in the 4 A series group of chicks which was fed the same Q-ration, as their peak parasitemias occurred on days five and six. Highest temperatures appeared on days four, five and six for the chicks of the 5 B series, whereas for the 5 A chicks given the same ration the highest temperatures occurred on the fourth day of the infection.

The chicks in the 6 A series (Tables 8, 18 and 28), as well as those of the 6 B series (Tables 19 and 29), were placed on the diet designated as S-ration on the fifth day of life. When they had reached the age of 13 days, the 6 A chicks were inoculated with P. lophurae, but the 6 B chicks were not inoculated with malarial parasites at any time. Half the chicks in the 6 A series died before reaching the tenth day of the infection, and those which did survive showed varying degrees of parasitemia on the tenth day from a low of 2.0 percent to a high of 26.0 percent. On the fourth day of the infection, nearly 60 percent of the red blood cells of the chicks were parasitized with P. lophurae. By the following day a high of 66.94 percent mean parasitemia was reached, and a high level was maintained during the days that followed. Most of the chicks which failed to show a material decrease in parasitemia died. Individual differences occurred among the chicks. The general trend was

a drop in the weight following the day of the highest parasitemia. Frequently this fall amounted to several grams. On the tenth day the mean weight for the 6 A chicks was only 102.5 grams. This was a contrast to the 6 B chicks fed the same ration but whose mean weight was 172 grams on the tenth day.

Data are presented in the form of graphs (Figs. 1, 2, 3, 4 and 5) to point out some typical patterns of parasitemia, temperature and weight changes which were followed by five chicks that had been inoculated with infective doses of P. lophurae. A sixth graph (Fig. 6) shows the weight and temperature changes of a chick which had not been inoculated.

Fig. 1 shows the pattern followed by chick No. 1461 of the 1 A series which had been given regular chick starting rations for five days, then transferred to the G-ration until inoculated when 12 days old with blood stages of P. lophurae. On the first day the 1 A chicks were weighed, when six days old, this chick weighed 60 grams, which was the heaviest for the lot. The mean weight for the group was 52.3 grams. By the time they were inoculated, chick No. 1461 weighed 82 grams, just five grams above the mean for the group. The growth curve appeared to be affected in some manner as this chick did not start to gain in weight very materially until the tenth day following inoculation, when just a few blood parasites could be demonstrated. A slight drop in the weight curve is shown for the day following inoculation. Another drop is shown for the day following the peak parasitemia.

The pattern of the parasitemia shows a continuous increase in percentage of red blood cells parasitized, with a slight increase between days five and six, and a sharp rise between days six and seven. The peak

parasitemia occurred on the seventh day of the infection. This was followed by a slight drop in parasitized cell count on the eighth day, and a sharp fall in number the ninth day.

On the day following inoculation, the temperature showed a very slight drop, but started to rise gradually on the next day, and continued to rise until the sixth day, which was the day prior to the peak of the parasitemia. On the day of the peak parasitemia there was a sudden drop in the temperature to the lowest point recorded for chick No. 1461. As the number of parasitized cells began to decrease in number, there was also an increase in the temperature. The temperature level attained was comparable to that which was recorded for the chick a few days before the peak parasitemia was reached. Chick No. 1461 was one of the chicks in which the eyelid lesions appeared. A description of this phenomenon occurs elsewhere.

Fig. 2 shows another pattern followed by a chick which had been started on the chick starting ration, but transferred to the G-ration when only three days old. This was chick No. 1622 from the 2 A series. When six days old, this chick weighed only 53 grams, which was 1.9 grams below the mean for the group. On the inoculation date, when the chicks were 12 days old, this chick weighed 76 grams, which was 1.6 grams more than the mean for the group. It seemed to hold its weight until two days following the day of highest parasitemia, then it lost 10 grams. However, as shown in the figure, there was a continuous rise in weight after the day of highest parasitemia. This was one of two chicks from the 2 A series that lived through the tenth day following the inoculation.

The curve showing the daily changes in parasitemia follows a pattern of relative increase in parasite number after the first day, followed by

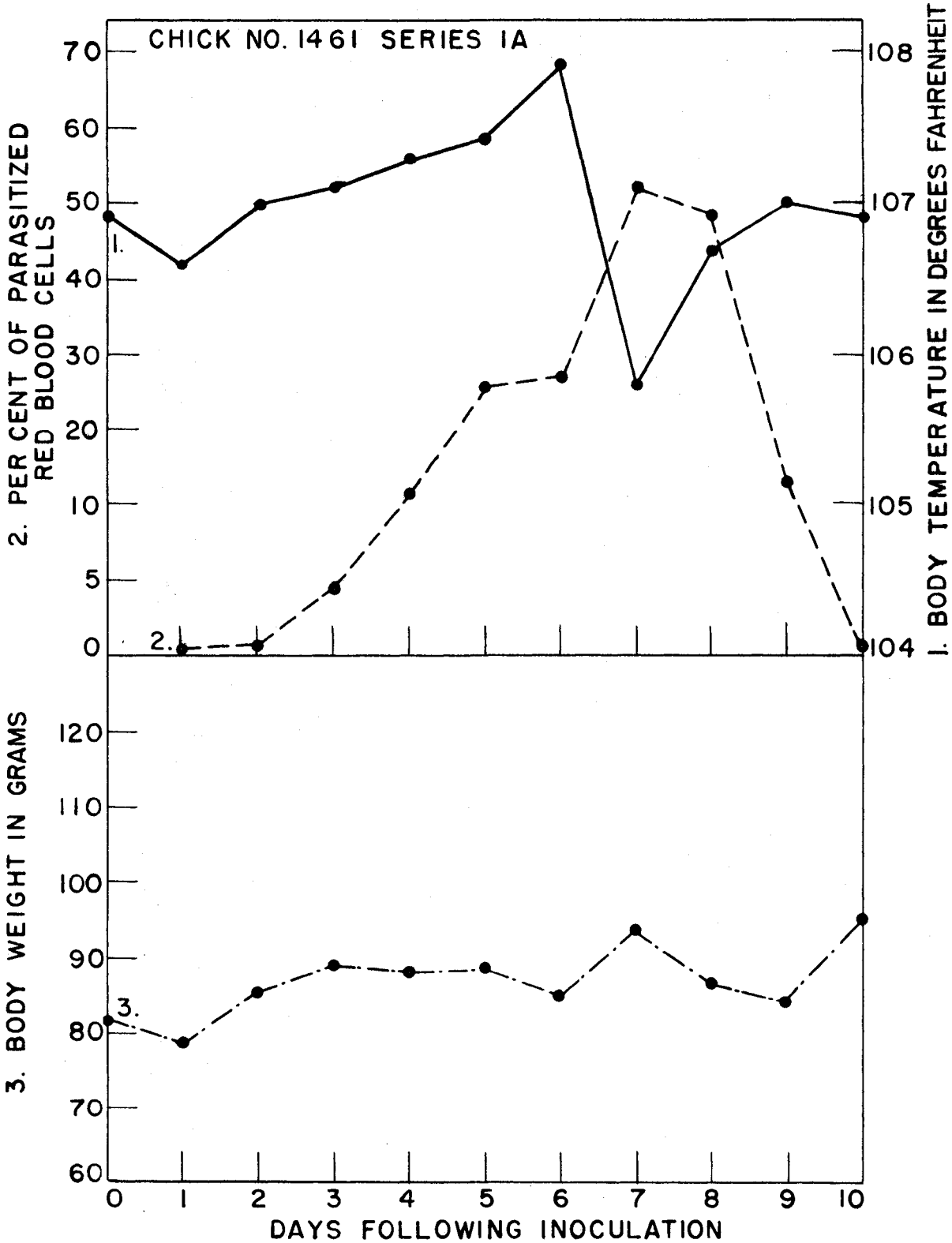

FIGURE 1. Pattern of temperature, parasitemia and weight followed by chick No. 1461 of 1 A series given G-ration.

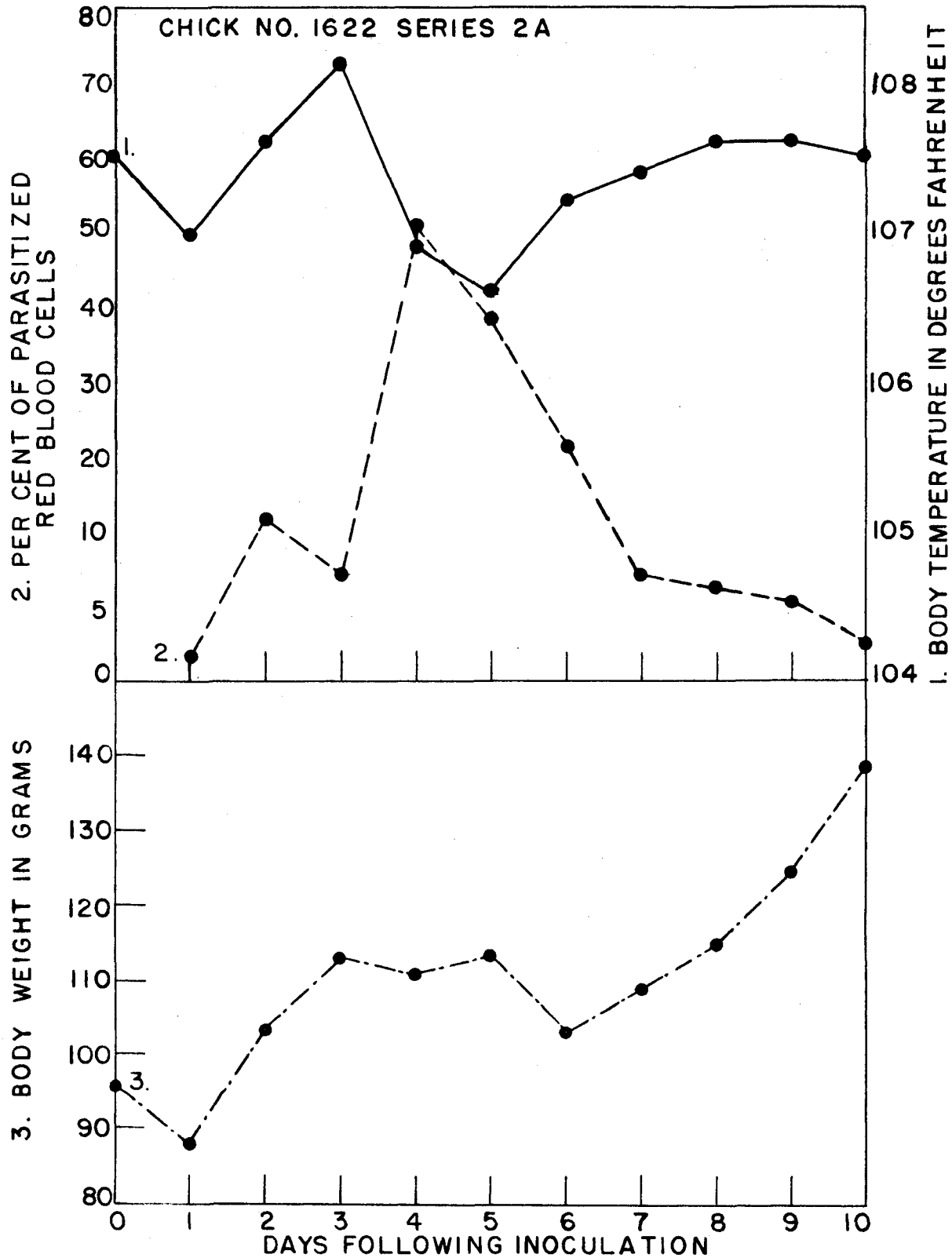

FIGURE 2. Pattern of temperature, parasitemia and weight followed by chick No. 1622 of 2 A series given G-ration.

a fall in number of parasitized cells, then a very great increase, with the peak parasitemia occurring on the fourth day following inoculation. The fall in parasite numbers was very rapid also, but not as rapid as the rise. After falling rapidly for a period of three days, on the fourth day the fall was much slighter. On the tenth day following inoculation, parasites were still microscopically demonstrable.

The temperature curve showed a slight fall on the day following inoculation, then a sharp rise until the day before the peak parasitemia was reached. Then a fall in temperature occurred on the day of the peak parasitemia, followed by a slightly less fall in temperature the next day. However, the temperature started to climb the second day following the peak parasitemia and reached levels comparable to those a few days preceding the peak parasitemia.

Fig. 3 shows the patterns of one of the 5 B series chick which had been placed on the Q-ration when five days old, and inoculated with P. lophurae when 12 days old. This chick, No. 13, which weighed only 60 grams when six days old, was right on the mean for the group. On the inoculation day it weighed 78 grams, which was 4.8 grams below that of the mean for the group. Its growth pattern was almost on a level as the weight changes per day were so slight. A slight drop in weight occurred as the parasite number in the blood began to climb, then again the day on which a peak parasitemia was reached, and again on the day following the tenth day, on which there was a second peak parasitemia. From that day on the chick lost weight steadily until it died on the thirteenth day following inoculation with P. lophurae blood parasites. When this chick died it weighed

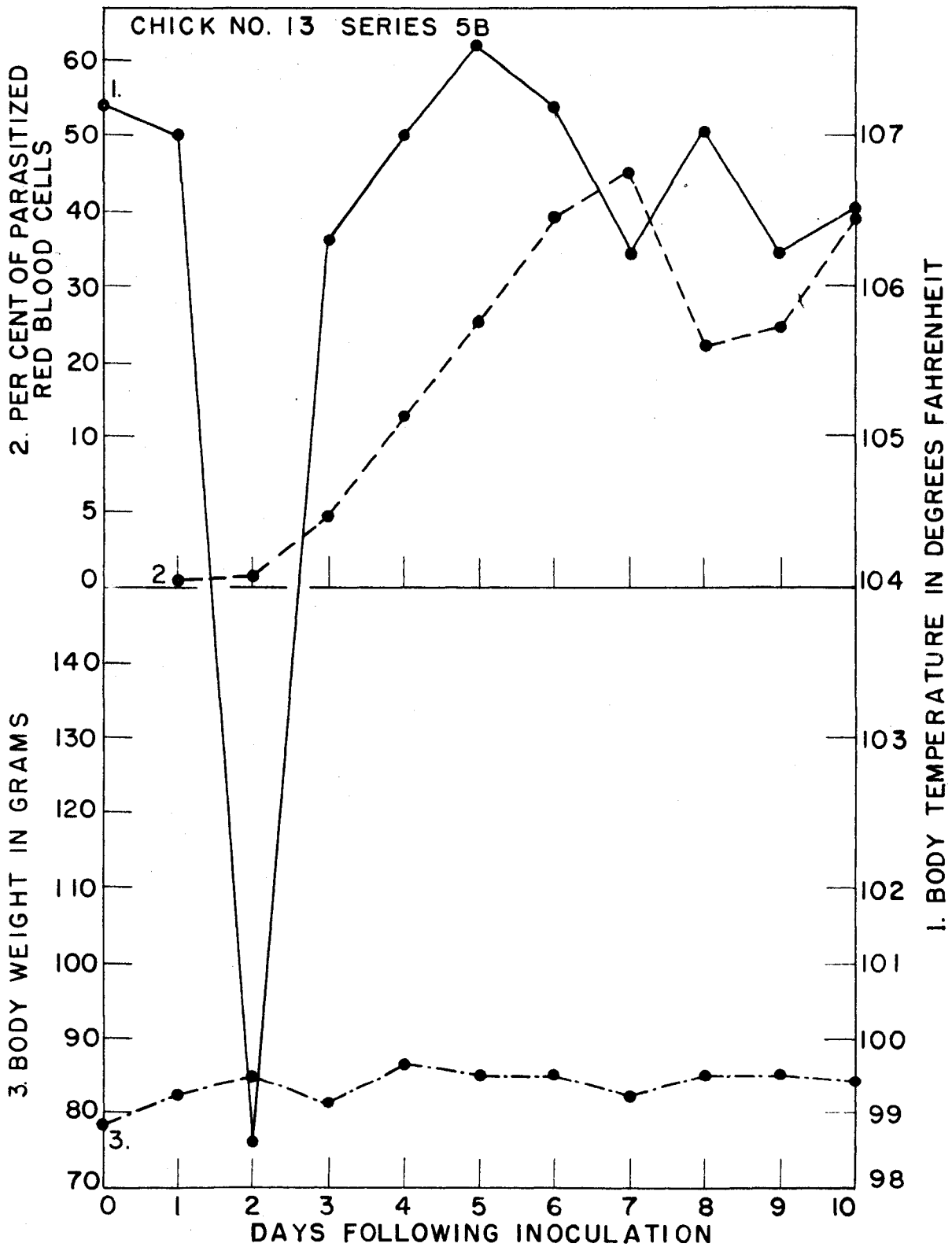

FIGURE 3. Pattern of temperature, parasitemia and weight followed by chick No. 13 of 5 B series.

only 72 grams which was six grams less than it had weighed on the day it was inoculated.

Two parasitemia heights were reached in chick No. 13. These occurred on days seven and ten following the inoculation date. The second peak reached a height of 39 percent, whereas the first was 45 percent. After the fourth day until the chick died, it showed not less than 22 percent parasitemia.

The temperature curve for chick No. 13 was interesting in that the second day following inoculation with P. lophurae, the temperature dipped to a low of 98.6° F. The next day it climbed up to 106.3° F. and reached a new high two days before the first peak parasitemia. A fall occurred until the peak parasitemia was reached, then the temperature again went up. The day before the second peak parasitemia was reached the temperature fell somewhat, but appeared to rise on the day of the second peak parasitemia. The day following the second parasitemia peak the temperature again dropped to a low of 103.1° F.

The profile of another chick of the 5 B series is shown in Fig. 4. This chick, No. 14, like No. 13, showed two peak parasitemias, but in this case the second peak was much higher than the first. The weight on the sixth day of life was 64 grams, which was four grams heavier than the mean. On the day of inoculation this chick weighed 90 grams, which was 7.3 grams above that of the mean. No drop in weight was exhibited on the day following inoculation. There was a continuous rise in the weight until the day before the first peak parasitemia was reached, then it fell slightly. The weight followed a course of slight increase, then decrease,

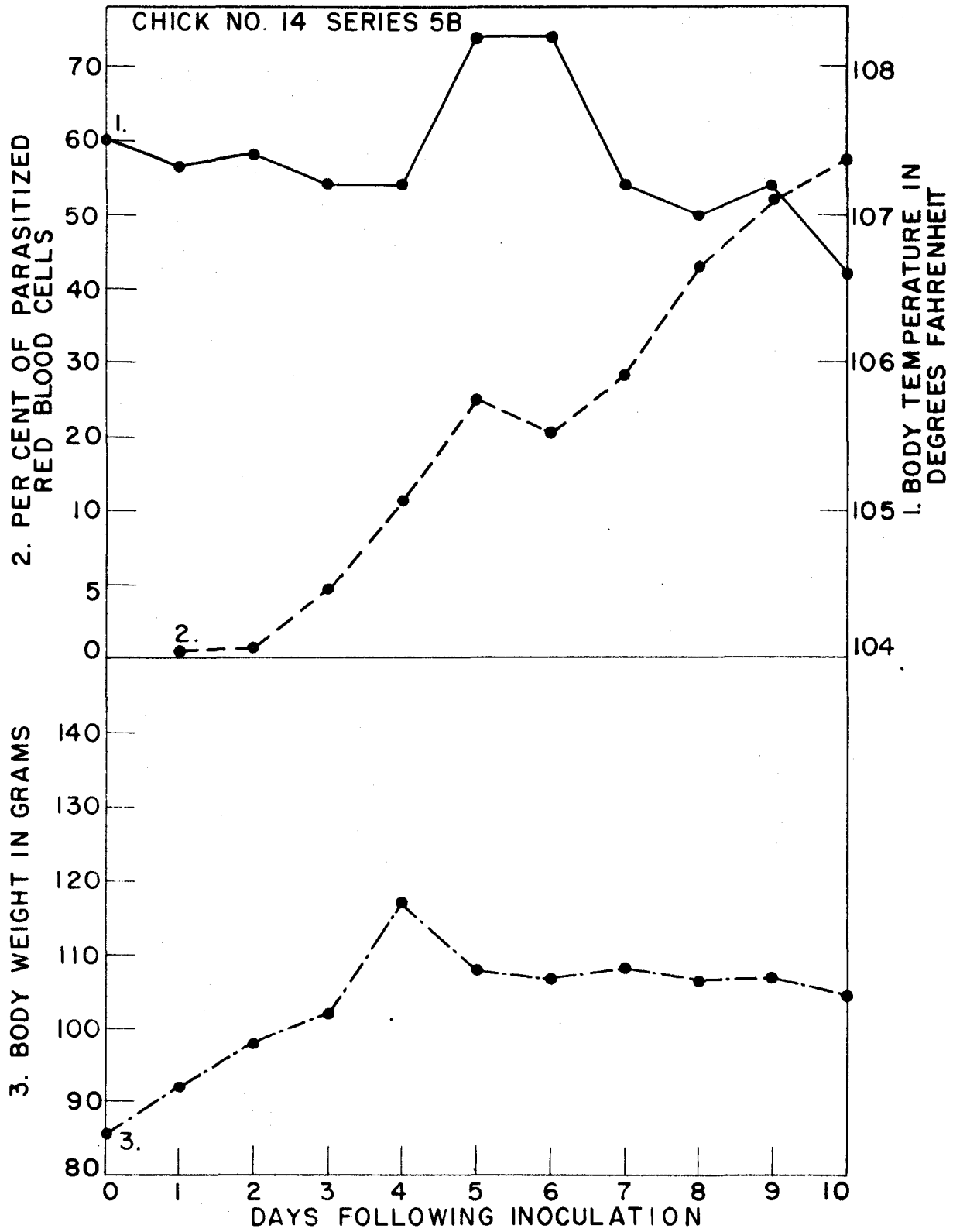

FIGURE 4. Pattern of temperature, parasitemia and weight followed by chick No. 14 of 5 B series.

until the chick finally died 17 days after it had been inoculated. At that time it weighed 104 grams, and appeared weak and sick. Before death, a few parasitized red blood cells could still be demonstrated in Giemsa-stained blood smears.

The first peak parasitemia was reached on the fifth day following inoculation, and the second was reached on the tenth day. The fall in parasitemia was very slow, with the parasitemia still at 46 percent on the eleventh day following inoculation.

The temperature exhibited a very changeable pattern, with first a slight fall on the day following inoculation, then irregular, slight rises and falls until the day of the first peak parasitemia (day five), when the temperature appeared at its highest point and remained there until the following day. This was followed by a drop, and a slight rise just before the second peak parasitemia was reached. Following the second peak parasitemia there was a sudden drop in temperature until it reached a low of 105.4° F. on the eleventh day. The low point of 102° F. was reached on the seventeenth day following inoculation after which time the chick died.

Fig. 5 shows the record of chick No. 1238 of the 6 A series. This chick had been placed on the S-ration after five days of being given the regular chick starting feed. Inoculation date was on the thirteenth day of its life. This chick weighed 65 grams when weighed at age of six days, which was 3.3 grams heavier than that of the mean of the group. On the inoculation date it weighed 90 grams, which was 9.1 grams heavier than the mean for the group. It was the heaviest chick in the group on the inoculation date. Its weight continued to increase until day four, the day before the peak parasitemia. The next two days it fell, gained slightly

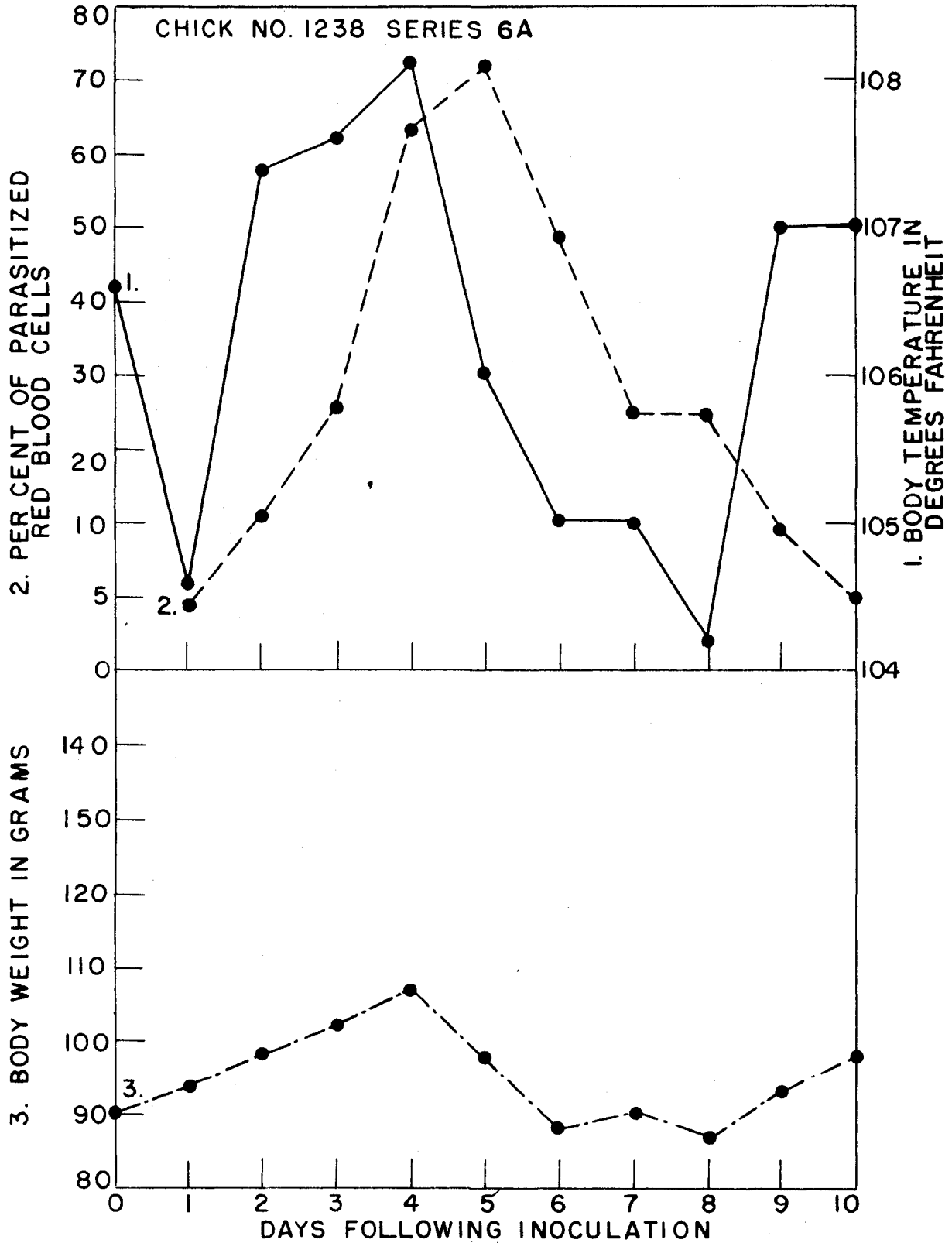

FIGURE 5. Pattern of temperature, parasitemia and weight followed by chick No. 1238 of 6 A series.

the next, then fell slightly again. Beginning with the eighth day this chick continued to gain in weight until on the twenty-first day following inoculation it had reached the weight of 338 grams, when it was killed.

The peak parasitemia of 72 percent occurred on the fifth day following the inoculation. For the two days following the peak the number of parasites in the blood fell rapidly, then from the seventh day on through the fourteenth the fall was less rapid.

The temperature pattern was very irregular. On the day following inoculation a sharp drop occurred, then an abnormally high temperature followed until a peak of 108.2° F. was reached on the fourth day, just before the peak parasitemia. The temperature fall was rapid from the fourth to the eighth day until a low of 104.2° F. was reached. The following day the temperature rose swiftly to 107° F. Temperature changes were less irregular until the chick was killed on the nineteenth day following inoculation.

Fig. 6 shows the weight and temperature records for an uninfected chick, No. 1231, of the 6 B series. It was given the S-ration at the same time as that given chick No. 1238. Weight on the sixth day of life was 72 grams. This was 9.5 grams heavier than that for the mean of the group. On the day when the other chicks were inoculated, this chick weighed 107 grams. This was 25 grams heavier than that for the mean of the group. The weight profile shows a steady increase in weight from the first day following inoculation of the chicks of the 6 A group, until this chick was killed when it was 21 days old. At the time it weighed 328 grams.

A somewhat irregular temperature pattern is followed. High points in the temperature were days four and five, with a regular falling in

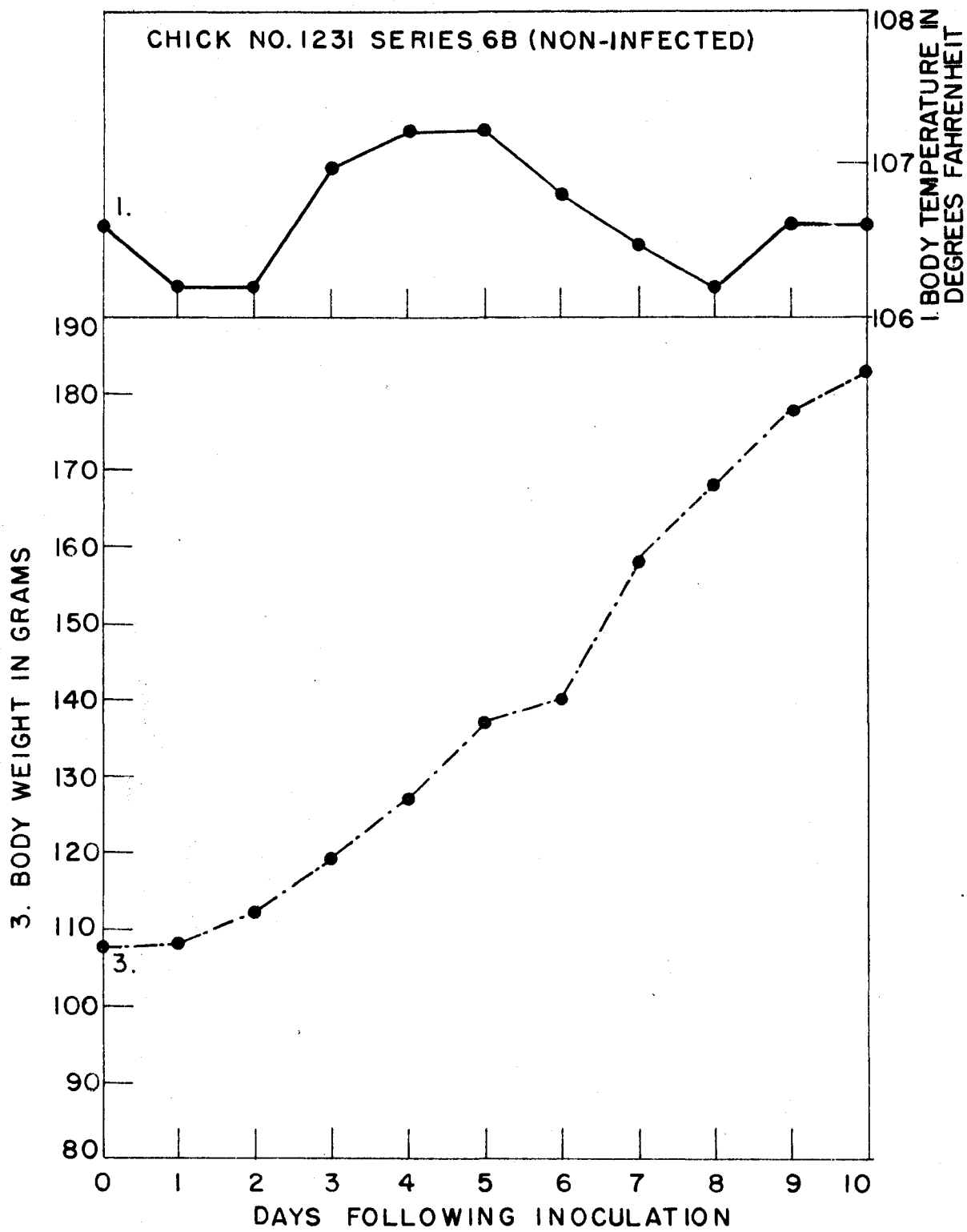

FIGURE 6. Pattern of weight and temperature followed by non-infected chick No. 1231 of 6 B series.

temperature from the fifth day until the eighth. After this, a slightly higher temperature occurred, with a more or less regular level following during the next few days.

There may not be any significant relationship between temperature changes and the parasitemia, but there appears to be at least some sort of a trend. Hewitt (1942) reported observations made on ducks and observed that subnormal temperatures usually preceded death, or followed high peaks of parasitemia in ducks of various ages and breeds. This same general pattern was observed in the present investigation for chicks. Frequently there was a high point in the temperature reading on the day preceding the peak parasitemia, with a fall in the temperature as the parasitemia was reached. In some of the infected chicks temperature drops to below 100° F. were observed before death.

In general, the daily temperatures of both the uninfected and the infected chicks showed some fluctuations. However, the sharp rises and falls observed in the infected chicks were not part of the general pattern for uninfected chicks. The range in temperature for the infected chicks was, in general, greater than that for the uninfected chicks.

Exoerythrocytic stages

Huff (1948) lists the species of Plasmodium for which phanerozoites have been found. The phanerozoites are exoerythrocytic stages which occur late in the malarial infection. James and Tate (1938) indicated they had observed a great deal of irregularity as to the time of appearance of the exoerythrocytic stages of P. gallinaceum. These stages appeared between the fifteenth and twenty-fourth day, or after the erythrocytic stages had

been cleared from the blood.

Huff and Coulston (1946), in their work with P. gallinaceum in chickens which had been inoculated with the blood forms of the parasites, reported that when phanerozoites did appear it was late in the infection, generally during the third or fourth week. This high development of phanerozoites during the time when the blood forms were practically or entirely absent from the circulating blood demonstrated the almost complete independence of the two components of the blood-induced avian malarial life cycle.

Becker and Manresa (1950) were the first to demonstrate the presence of phanerozoites in turkeys which had been infected with the blood stages of P. lophurae. They were found in the brain capillaries of two 46-day-old turkeys which had succumbed on the twenty-first day of the infection. Examinations of smears prepared from the spleen, liver, kidney, heart and lung of these turkeys, as well as from others, failed to show the presence of the phanerozoites.

For the present investigation, Table 31 lists the various organs together with the total number of each kind of organ examined for the presence of phanerozoite forms of exoerythrocytic stages of P. lophurae. Organs were removed from the host birds soon after death or after they were sacrificed, from the third through the ninety-fifth day following inoculation.

Birds of the 1 A and the 1 B series which died during the investigation generally did so within a very few days after inoculation. Blood smear and tissue examinations indicated that many of the red blood cells were parasitized, but showed no exoerythrocytic forms to be present.

Chicks that continued to survive following inoculation and early parasitemia were still living a month after inoculation had occurred. Those which were sacrificed failed to show the presence of either blood stages or exoerythrocytic stages of P. lophurae.

Six of the ten 2 A chicks were dead by the time the day of inoculation with P. lophurae parasites had arrived. Of the remaining four, one lost weight steadily and finally died on the sixth day following inoculation. An examination of its tissues failed to show the presence of exoerythrocytic stages. A chick which died on the fourth day following inoculation likewise failed to show exoerythrocytic stages in the blood or in the tissues. In each of these two chicks, a macroscopic examination of the organs showed that the gall bladder was greatly distended and the liver was dark. Both chicks appeared to be weak and sick just before death. The other chicks were sacrificed on the twenty-fifth day following inoculation. Examination of blood smears and slides of tissues revealed neither blood forms nor exoerythrocytic forms. An explanation for the early death of six of the ten chicks before they were inoculated may have been due to the very early date (third day) that these chicks were placed on the G-ration.

The chicks of the 3 A series which survived the first seven days following inoculation continued to live until they were killed two weeks later. Examinations of tissues revealed the presence of neither erythrocytic nor exoerythrocytic stages of P. lophurae.

Chicks of the 4 A series that died appeared to suffer from dietary difficulties at the time of their death. Enlarged gall bladders and a

watery appearance of the internal organs were common occurrences, but no exoerythrocytic stages of P. lophurae were observed.

All of the chicks except one of the 5 A series survived through the first 16 days following inoculation with P. lophurae. This chick which died (No. 8) was found to have had a continuously higher than normal parasitemia than the other chicks from the third day following inoculation until its death on the sixteenth day following inoculation.

No exoerythrocytic stages were observed in the chicks of the 6 A series. Of the ten chicks in the 1 A series, six had died by the tenth day following inoculation, and a seventh was so weak that it was killed. All the chicks of this group had demonstrable parasitized red blood cells present in the circulating blood on the tenth day following inoculation, but no exoerythrocytes were observed. Blood parasites varied in density in the different chicks.

Phagocytosis

Stained sections, smears and imprints of organs were examined microscopically for the presence of cells which showed phagocytosis of parasitized red blood cells and malarial pigment. Day of the infection of the chick hosts examined varied from three days after inoculation with P. lophurae through the ninety-fifth day. Names of the organs which were examined for phagocytosis response are shown in Table 31.

It was observed that the responses of individual chicks varied greatly insofar as the phagocytosis of foreign elements is concerned. Cerebral tissue of a chick that had died the third day following inoculation showed some malarial pigment to be present in the form of

scattered granules. A few parasitized red blood cells were likewise present in this tissue. Blood smears showed a parasitemia of only 2.3 percent. In a chick that died on the sixth day of the infection all degrees of parasitism of the red blood cells were demonstrated. Three and four parasites inside one red blood cell was not an uncommon occurrence. The cells containing these parasites were observed in the brain capillaries.

There seemed to be a general response of the leucocytes to the presence of the parasites as shown by the increased number of leucocytes observed in the brain tissue. Pigment still appeared to be present in the form of small granules in the brain. The brain smears were better for observing the pigment. In the chicks which had reached the ninety-third and the ninety-fifth days, respectively, following inoculation, malarial pigment no longer appeared to be present.

Lungs. Observations of the lung tissue were also made three days following inoculation. Although there were no parasitized red blood cells demonstrated in the lungs, there was a great deal of scattered pigment present. Some of it appeared as small individual granules, but for the most part it appeared as clumps of pigment, usually in the tissues just adjoining the air spaces.

Fig. 7 shows the appearance of clumps of the pigment scattered throughout the lung tissue. In two chicks which had died five days following inoculation, pigment was observed in the lungs, but was not present in the form of such large clumps as that of the chick with the three-day infection. In chicks with older infectipns up to 14 days small granules and large clumps

of pigment were observed. Pigment was not observed in the lungs of the chick with the 95-day-old infection.

Spleen. The spleen was one of the best organs for demonstrating the presence of pigment. In the three-day-old infection, pigment granules were scattered over the entire spleen. In some areas the granules were clumped together in masses. Macrophages showed ingestion of pigment throughout the entire areas observed. Some of the pigment appeared to be free in the area and not inside the cells. Whether the slide was of a smear, an imprint or of a section did not appear to make any difference as to the appearance of the pigment. According to Boyd (1949) the greatest phagocytic activity occurs in the Billroth cords of the spleen where the blood flow is slowed down and the most active phagocytic cells are concentrated. The parasitized red blood cells observed in the macrophages of the spleen appeared to be degenerating. A few instances were noted in which the phagocytic cells actually appeared to be ingesting a parasitized red blood cell. Another common observation made of the spleens that were examined macroscopically was their enlargement. When compared with non-infected chicks of the same age and observed on the same day, they were one to two and one-half times the volume of the non-infected spleens. The pigment masses were observed in chicks as late as the thirty-first day following inoculation. Spleens of the chicks killed three months after inoculation showed scattered pigment granules, but not the huge masses observed in earlier infections. Fig. 8 shows the masses of pigment present in the spleen of a chick which had died three days after inoculation with blood forms of P. lophurae.

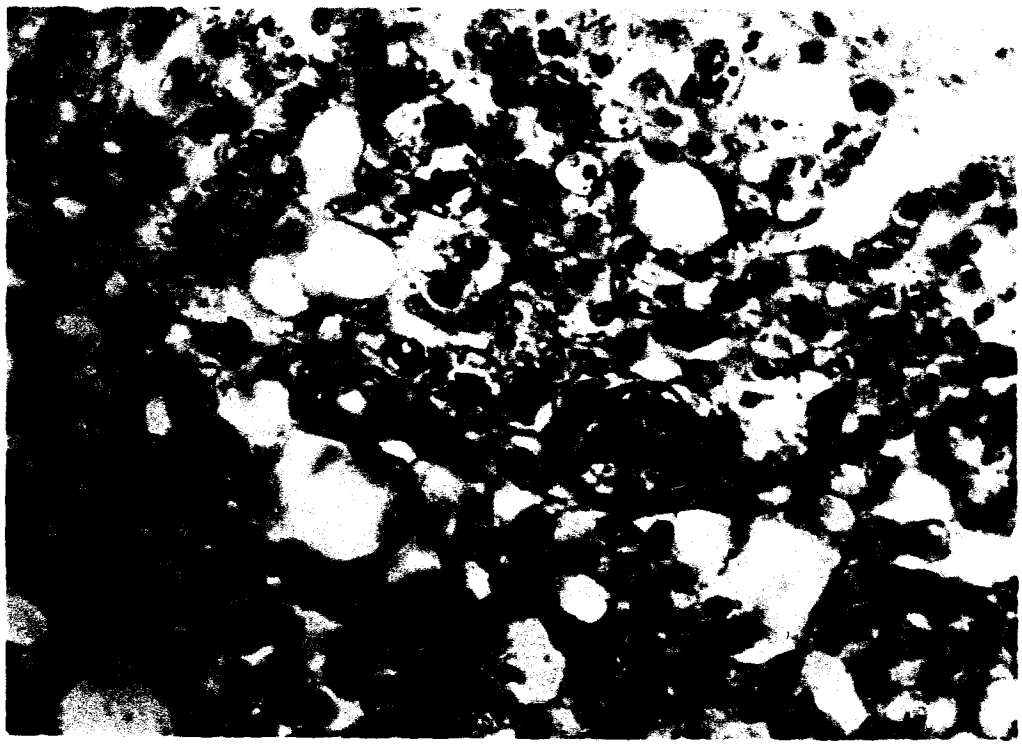

Fig. 7. Section of a lung showing malarial pigment on the sixth day of the infection from chick No. 1619, series 2 A.

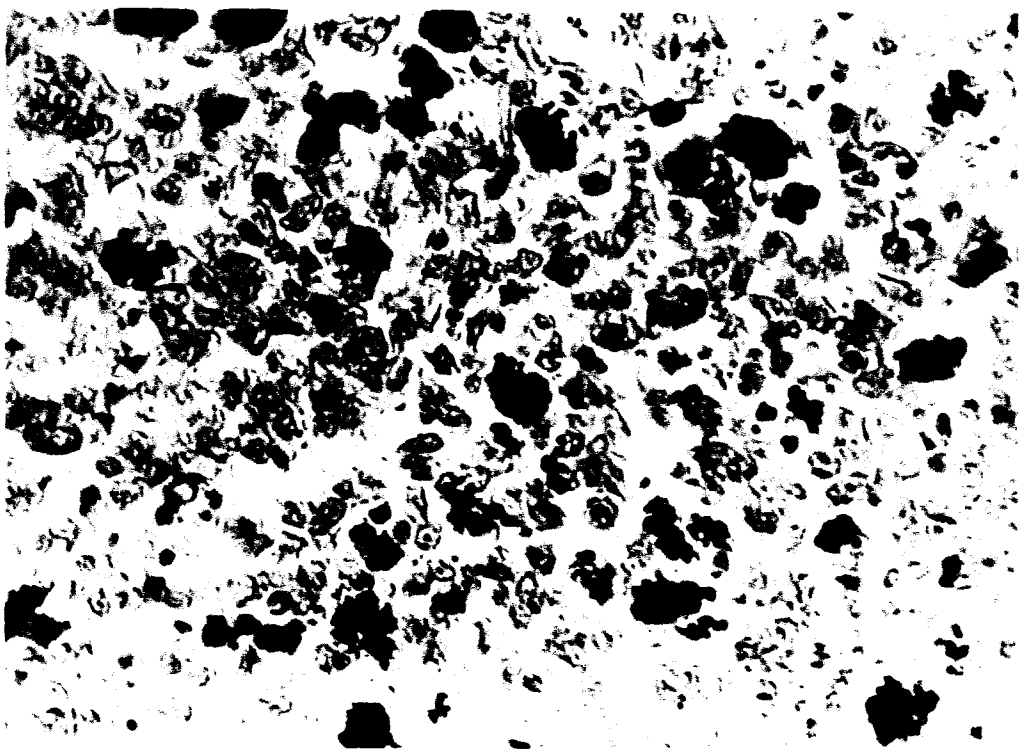

Fig. 8. Section of spleen showing malarial pigment on the third day of the infection from chick No. 1456, series 1 A.

Kidneys. Masses of malaria pigment appeared in the kidneys on the third day following inoculation. Connective tissue between the kidney tubules sometimes contained large masses of the pigment. Figure 9 shows a section of kidney from a chick with a three-day infection in which the pigment is in the connective tissue between the tubules. Frequently the kidneys showed some degeneration when they were stained. The pigment disappeared from the kidneys about the second week of the infection.

Liver. Chicks with three-day infections showed macrophages scattered throughout the liver and packed tightly with parasitized red blood cells containing pigment. The number of parasitized red blood cells present in the macrophages varied. For later infections, the amount of pigment present varied considerably in different chicks. When examined macroscopically the liver and the gall bladder were generally enlarged and dark colored. As in the other tissues, the appearance and the distribution of pigment were individual responses. Phagocytosis of all stages of development of the parasites within the red blood cells occurred. By the time the infection had reached 31 days, most of the pigment had disappeared from the liver. The dark appearance of the liver was a constant feature of the malarious individual. Figure 10 shows the appearance of some of the masses of pigment and some granules from the liver of a chick that died three days following inoculation.

Adrenals. Some phagocytosis of pigment and of parasitized cells appeared in the adrenals. This occurred early and disappeared by the end of two weeks. When pigment did appear, it was in the form of rather small granules.

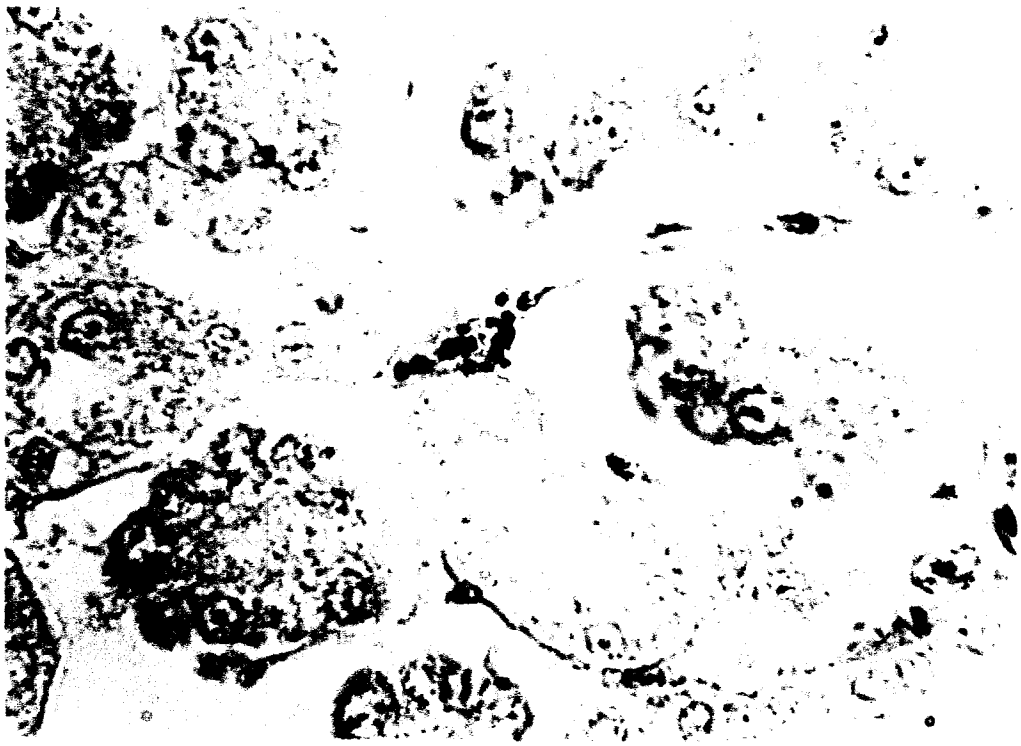

Fig. 9. Section of kidney showing malarial pigment on the third day of the infection from chick No. 1456, series 1 A.

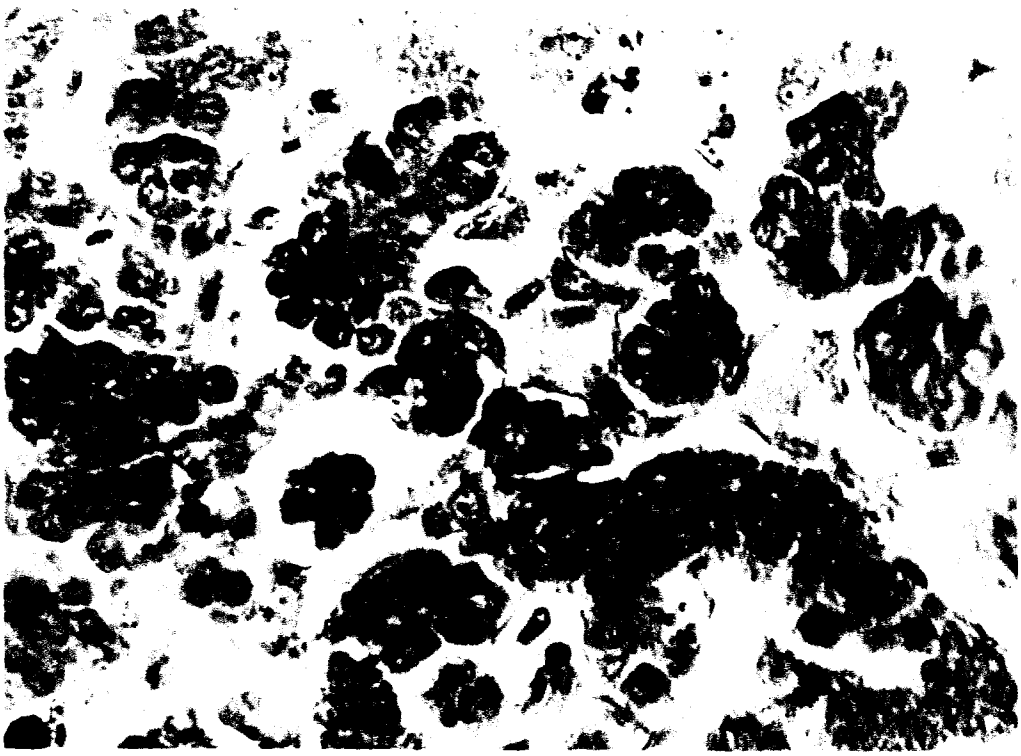

Fig. 10. Section of liver showing malarial pigment on the third day of the infection from chick No. 1456, series 1 A.

Gonads. Early in the infection a few granules of pigment were observed in the interstitial cells of the gonads, but these disappeared early. By the tenth day following inoculation gramules of pigment were no longer observed.

Eyelids. Deposits of very small amounts of pigment were observed in a few of the eyelids examined. In a 13-day infection a few infected red blood cells could be seen in the eyelid region.

Bone marrow. A few granules of pigment were observed in the bone marrow during early stages of development of the infection. With their appearance, there appeared to be an increase in the number of leucocytes.

Heart. Phagocytosis was not seen in the heart tissue, although parasitized red blood cells were seen.

Pancreas. Pigmentation of the pancreas did not occur in any of the chicks.

Other tissues. In sections of organs such as the stomach, rectum and crop, no phagocytosis appeared.

Appearance of Eyelid Lesions

The chicks which were kept on each of the rations, but particularly those given the designated G-ration, were closely observed for the appearance of the eyelid lesions described by Becker, Brodine and Marousek (1949). According to these authors the microbiological assays of the biotin and pantothenic acid content were made by members of the Department of Foods and Nutrition at Iowa State College. For assaying the biotin content a sample of the G-ration was autoclaved with 4 N sulfuric

acid for two hours. Lactobacillus arabinosus was the test microorganism used. The results indicated that 0.21 mg. of biotin was present per gram of G-ration. For the pantothenic acid assay, the sample was treated with Mylase P. incubated at 37° C. for 24 hours, and then autoclaved. The test organism used for this assay was Lactobacillus casei. The pantothenic acid content was found to be 20.1 mg. per gram of the G-ration.

Chicks of three of the test groups were given the G-ration. Ten of the chicks in the 1 A series were given the regular chick starting ration for five days before they were placed on the G-ration. They were kept on this ration for seven days, then inoculated with P. lophurae blood stages when they were 12 days old.

Chicks of the 2 A series were started on the regular starting diet, then transferred to the G-ration on the third day and, when 12 days old, they were inoculated in the same manner as the chicks in the 1 A series.

Chicks of the 3 A series were kept on the regular diet for five days, then placed on the G-ration for a period of eight days. At the age of 13 days they were inoculated with P. lophurae, as were the others.

Observations of the three groups were not made concomitantly, as the chicks were brought to the laboratory at different periods of time. Eyelid lesions appeared among four of the chicks of the 1 A series.

Three of the four chicks of the 4 A series which were given the G-ration showed early symptoms of a sensitivity of the eyes on the fourth day following inoculation. None, however, developed the actual lesions observed in the chicks given the G-ration. One of these chicks, No. 1844, had the left eye swollen shut on the eighth day following inoculation, but by the ninth day it appeared that no lesion would develop.

Forty percent or four of the ten chicks of the 1 A series showed varying degrees of intensity of the eyelid lesion phenomenon. The description of the lesions of both eyes observed in the chick designated as No. 1460 of the 1 A series points out the symptoms and general pattern followed by all the chicks in which lesions were observed.

On the sixth day following inoculation with P. lophurae the right eye of chick No. 1460 appeared to be very sensitive to light. The chick avoided the side of the cage near the light and hovered in the darkest corner. When brought to the light, it closed both eyes and turned its head to the right, appearing to shield the right eye from the light. When placed back in the pen it again sought the dark corner. Toward night the right eye was very watery and even more sensitive than earlier. By this time the left eye was showing the same signs as the right had shown earlier. The next morning the right eyelid was swollen shut and the left eye was very watery and the lid partially closed. Both eyes became festered with the exudate forming a crust over the entire eye by the ninth day. During this time the chick fed but little and its weight dropped from 90 down to 80 grams during the period from the sixth to the eighth day. The following day the scab came off leaving a ragged-looking margin about the eyeball. However, the chick started to gain weight and a new growth of tissue began to develop rapidly in place of the eroded eyelid. This tissue appeared to be considerably thicker than the original lid and it nearly covered the eyes. A tiny circular "peep-hole" was left in the right eyelid and a small slit was formed in the left. During the time that the new growth and eyelid thickening process occurred, the other chicks in the pen were seen to peck at the tissue frequently. The

chick suffering from the affliction constantly scratched the lids alternating the scratching with first one foot and then the other. After about five days of the constant scratching, it suddenly stopped. Chick No. 1460 was permitted to live for nearly three months at which time its weight was but slightly lower than that of other chicks the same age, whose diet was normal and who did not suffer from the eyelid lesions.

Differences as well as likenesses appeared between the eyelid lesion phenomenon described by Becker, Brodine and Marousek (1949) and those indicated in the present research. In the earlier work it was reported that the lesion symptoms appeared on the fourth or the fifth day, but in the present research they appeared on the sixth or the seventh day.

Parasite dosage was greater for chicks in the earlier work than in the present. Dosage ranged between 1×10^8 and 2×10^8 chick cells with parasites per 100 grams of body weight in the earlier experiments as compared with 0.8×10^8 chick cells with parasites per 100 grams of body weight for the infective dose for the present research.

None of the chicks of the present investigation attained a parasitemia which exceeded 50 per cent except No. 1461, which had 52 percent of its red blood cells parasitized on the seventh day after the inoculation with P. lophurae. A rapid rise in parasite count was not a constant feature of the present work which seemed to be characteristic of the earlier work. Like the earlier work, the lesions appeared only among chicks which were given the G-ration. Symptoms, course of development and end results were similar in the two studies. Early symptoms appeared but eye lesions did not materialize in three chicks given the Q-ration.

A prominent notch was left in the lower lid of some of the chicks that suffered a less severe lesion, in both the present and the earlier works.

The reasons as to why the eyelid lesions appeared among some chicks and not among others given the same ration are not fully understood. In the explanation of the development of biotin in foods, the work of Wright et al. (1941) may offer one clue. He points to the development of biotin in foods over a period of time. As biotin is developed by microorganisms in food, it is possible that immediately following the mixing of the rations in the laboratory the biotin content was not as high as after the food had remained in the laboratory for some period of time. This may account for the presence of eyelid lesions in the chicks given the G-ration shortly after the food was mixed and not in the chicks which were later given the same ration after it had stood for awhile in the laboratory. However, as all the chicks in the first experimental group did not develop the lesions, possibly some of these were unable to use the biotin which was available to the extent that the rest of the group did.

Development of biotin may at least in part account for the slight symptoms of weak and sensitive eyes observed in the first group of chicks given the Q-ration, and in none of the chicks brought to the laboratory later. The chicks of the 4 A series were the first ones to be given the freshly mixed Q-ration which was made up in part of the G-ration. None of the chicks brought to the laboratory later observed any symptoms of lesions.

The ability of some chicks to use biotin may be greater than in others. This may be tied up with the general metabolism of the chick.

Apparently the number of parasitized red blood cells present does not cause the lesions to appear, as the chicks which showed the highest parasitemia were not always the ones to develop the lesions.

Table 32 shows the ration given each of the groups of chicks observed in the present research, and points out the mean percent of parasitized red blood cells which appeared in each of the groups during the period when the development of eyelid lesions might be expected. The groups are listed in the order in which they were observed in the laboratory.

Ducks

Parasitemia and weight changes

A presentation of the parasitemia and the weight changes of the ten ducks which were inoculated with exoerythrocytic forms of P. lophurae from an infected turkey are shown in Tables 9 and 30. Blood stages of P. lophurae were present in the red blood cells of the ducks on the fifteenth day following inoculation. On that day parasitized red blood cells appeared in Giemsa-stained blood smears from six of the seven ducks which had not as yet been sacrificed. One duck was sacrificed each day from the fifteenth day until all the ducks were killed. Parasitized red blood cells were demonstrated only through the eighteenth day. The highest parasitemia for any of the ducks was 5.67 percent, demonstrated in duck No. 46 on the eighteenth day following inoculation. The day before the ducks were inoculated they were weighed and the mean weight was found to be 56 grams. On the fifteenth day two ducks were found to weigh more than 500 grams

apiece. After that weighing of the ducks was discontinued. The ducks appeared to be in good physical condition during the entire time they were under observation. The low grade of parasitemia which developed in six of the ten ducks did not appear to cause any difficulty insofar as weight increases were concerned as indicated by the table showing daily weight changes in the ducks.

Exoerythrocytic stages

Since the ducks were inoculated with exoerythrocytic stages from turkey brains, it might be expected that exoerythrocytic stages would develop from them, if at all. That did not happen, however. Ducks were killed from the twelfth day until the twenty-first day after inoculation. Blood parasites were demonstrated on the fifteenth through the eighteenth day, but no exoerythrocytic stages could be observed. Since many of the exoerythrocytic stages are known to develop rather late after the blood parasites have been cleared from the blood, it is barely possible that, if the ducks had been permitted to live longer, some of them might have exhibited the exoerythrocytic stages.

Four elongated, irregular bodies were found in the duck brain smears that had been stained with Giemsa. They were located outside the brain capillaries and measured from 41.4 microns to 46.4 microns in length, with the widest diameters from 14.0 to 22.2 microns. Each was stained a rather bright blue with more than 100 small, dark, purple-red figures within. These varied in diameter from .43 to 1.74 microns. No attempt is made to explain these unusual bodies other than to indicate the possibility of their being some form of exoerythrocytic stage. They were present at about

the time one might expect exoerythrocytic stages to occur, but were found in only one duck. Further investigation using more host birds might shed some light on the identity of these bodies.

Phagocytosis

The tissues of the ducks showed very slight concentrations of the presence of malarial pigment and in many no pigment could be demonstrated in the tissues. In the lung tissue, there was only one dusk for which any pigmentation was observed. This was a 16-day-old duck in which there were a very few pigment granules scattered through the lung tissue. Phagocytosis of a few granules of pigment appeared in three of the ducks and could be observed on days 12, 13 and 14 before any parasitized red blood cells were demonstrated in the circulating blood. One duck showed phagocytosis in the spleen on the twelfth day. In the kidneys there was increase in the number of basophils on the fourteenth day, and occasional granules of pigment could be observed scattered over the kidneys between the tubules in the interstitial tissue. Slight pigmentation of the liver was observed from the sixteenth day on through the twenty-first day of the infection. Heterophils appeared in the spleen and liver from the twelfth day on. No pigment was observed in the heart tissue. Slight amounts of pigment granules appeared in the brain tissue on the fourteenth day.

Turkeys

The two turkeys which were used in the investigation had been

inoculated with infected red blood cells from ducks and had demonstrated the presence of the blood stages of P. lophurae. One of the turkeys died on the twenty-second day following inoculation and the other was sacrificed on the thirtieth day following inoculation. In the one which died, exo-erythrocytic stages appeared in the brain capillaries. They were similar to the ones described by Becker and Manresa (1949) and Manresa (1950). In addition, some parasitized red blood cells were observed in the brain capillaries. A few parasitized red blood cells were also observed in the kidney and lung. Malarial pigment was seen in the spleen, lung, liver and adrenals. In the turkey which had been killed, no evidence of exoery-throcytic stages of P. lophurae were demonstrated. Neither were there demonstrated any of the blood forms of P. lophurae. However, pigment was scattered throughout the spleen tissue in the form of large clumps. Malarial pigment was also observed in the liver in rather large amounts. It was not seen in other organs.

SUMMARY AND CONCLUSIONS

The present investigation was undertaken to determine some of the responses which domesticated birds make to the malarial parasite, Plasmodium lophurae, under various conditions. Among the responses observed were (1) parasitemia, (2) effects of different rations on the host, (3) temperature changes in groups and in individual birds, (4) changes in weight, (5) eyelid lesions, (6) phanerozoite stages of the malarial parasite, (7) phagocytosis and appearance of malarial pigment in the various organs and (8) a general summary to show comparisons of all groups.

Parasitemia

Chicks

Parasitized red blood cells were observed in the blood smears of all the chicks that received blood from passage chicks. Apparently the dosage of 0.8×10^8 parasitized red blood cells per 100 grams of body weight of chick produced different levels of parasitemia in different chicks. Ranges varied from a peak parasitemia of 12.75 percent on the eighth day of the infection to 100 percent on the fifth day of the infection. Results of parasitemia are shown in Tables 1 through 8, and Figures 1 through 5.

Ducks

A dosage of 0.9 cc. of an emulsion of turkey brain tissue infected with phanerozoite stages of P. lophurae diluted 1:20 in saline was great

enough to produce blood stages of the malarial parasite in seven of the ducks inoculated with it. It was not great enough apparently to produce severe parasitemia in the ducks. Neither did it produce exoerythrocytic stages of P. lophurae up through the twenty-first day following inoculation. Results of parasitemia are shown in Table 9.

Turkeys

Two turkeys which had been inoculated in the same manner as the chicks except that parasitized red blood cells from a passage duck were used instead of parasitized cells from a passage chick, received a dosage sufficient to produce blood parasites of P. lophurae. In addition, phanerozoite stages developed in one of the poults.

Effects of Different Rations on the Host

A comparison of the chicks on the day of the inoculation with that on the tenth day following inoculation as shown in Table 33 points out the groups which appeared to fare best on the rations provided them. Non-infected chicks on the S-rations and non-infected chicks on the regular rations appeared to show the best results, in that these chicks followed a continuous pattern of growth and their temperatures showed less variation. In addition they presented a sturdy and healthy appearance. When their internal organs were examined macroscopically they were firm. The liver and spleen were not dark as was true of many of the infected chicks that died or were sacrificed.

Temperature Changes of Groups and of Individual Birds

Results of the temperature investigation are shown in Tables 11

through 19, and in Figures 1 through 6, for both the infected and the non-infected birds. Variations from 97° to 109.4° F. occurred in the infected chicks, while temperature readings from 99° to 107.9° F. occurred in the non-infected birds.

Daily Changes in Weight

Tables 20 through 29 show changes in weight observed in chicks. Table 30 shows changes in weight for ducks. Table 33 makes a comparison of the various groups of chicks given the different diets on the day of inoculation and the tenth day following inoculation. Mean gains for each group are shown. Figures 1 through 6 show individual weight records for chicks selected from different groups.

Appearance of Eyelid Lesions

Eyelid lesions appeared in one group of chicks which were given the G-ration. Slight symptoms were noted in some of the chicks given the Q-ration. It is possible that a lack of biotin may be a factor in the appearance or non-appearance of the lesions. Figure 32 gives a summary of all the groups of chicks and the occurrence of the phenomenon.

Appearance of Phanerozoite Stages

Results of the investigation of the various organs in chicks, ducks and turkeys for phanerozoite stages are presented in Table 31. Names and the number of each organ observed are listed. No phanerozoite stages were observed in the chicks, ducks and one turkey. The only phanerozoites that could be demonstrated were found in the brain capillaries of one

turkey which had been inoculated from a passage duck. The description of four elongated, irregularly-shaped bodies occurring in one duck is given under the results in phanerozoite investigation in ducks. As the appearance of these bodies does not conform to the classical description of phanerozoites, further investigation should be made before results are finally determined.

Phagocytosis and Appearance of Pigment in the Various Organs

Table 31 is presented to show the occurrence of phagocytosis and malarial pigment observed in the organs of the host birds examined. Figures 7 through 10 show appearance of malarial pigment and of phagocytosis occurring in four of the organs of chicks which died following inoculation. Clumps of pigment as well as small granules were seen in all stages of the infection.

General Summary

Tables 34 and 35 are presented as a general summary to show a survey of the investigations on the different groups of chicks. It was observed that the group showing the highest mean weight on the day of inoculation did not necessarily develop the highest parasitemia. Neither did the group with the lowest mean weight develop the lowest parasitemia. This appeared to be an individual response of the chick. Highest day of mean parasitemia varied with the individual groups. Group variation ranged between days five through eight of the parasitemia. The day on which the highest mean temperature occurred, appeared to be related to the day of highest

mean parasitemia, in that the highest mean temperature so frequently occurred on the day prior to the highest mean parasitemia. Mean temperature for the group usually dropped to below previous levels on the day of the highest mean parasitemia. The highest mean temperature, 108.1° F., for all the chicks occurred in the 4 A chicks given Q rations and the 5 A chicks given R rations. The lowest mean temperature of 107.1° F. occurred in the non-inoculated chicks of the 6 B series.

Table 35 gives a summary of the birds in each group which were examined and which showed positive results for each of the following: (1) the presence of parasitized red blood cells; (2) eyelid lesions; (3) phagocytosis; (4) malarial pigment; and (5) phanerozoites.

1. in 84 of the 87 birds examined the presence of parasitized red blood cells was demonstrated. The only birds which were microscopically negative were three ducks which had been inoculated with phanerozoite stages from a turkey brain.

2. Four chicks from the 1 A series showed definite presence of eyelid lesions, and three of the 4 A series showed slight symptoms. No other birds from any group showed symptoms of eyelid lesions.

3. Phagocytosis either of parasitized red blood cells or of malarial pigment occurred in macrophages in 49 of the 62 birds examined. Birds showing positive results included 43 of the 50 chicks examined, five of the 10 ducks and one of the two turkeys. Organs showing most active phagocytosis were the liver, kidneys, spleen and lungs.

4. Malarial pigment was observed in 59 of the 62 birds examined. This included all of the 50 chicks, seven of 10 ducks and both turkeys examined. Pigment occurred in the form of small granules scattered over

the cut surfaces of the organs or in masses and clumps in the various organs.

5. Only one bird exhibited phanerozoite stages, and it was on the twenty-first day following inoculation. Fifty chicks, ten ducks and two turkeys were examined for phanerozoites. The phanerozoites observed were in the capillaries of a brain smear of a turkey which had died twenty-one days after being inoculated with P. lophurae blood forms. No organs other than the brain were found to have the phanerozoites present. The four irregularly shaped, multinucleate bodies observed in the brain of a duck killed on the twenty-first day following inoculation do not conform to the classical descriptions of phanerozoites so should be further investigated before definite conclusions are reached concerning their status. They may be mere artefacts, or they may represent phanerozoites developing aberrantly in an unfavorable host.

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APPENDIX

Table 1. Percentage of parasitized red blood cells present in infected 1 A series chicks during the ten-day period following inoculation with blood stages of P. lophurae from a passage chick. (Parasite dosage, 0.8 x 10^6 parasitized cells per 100 grams body weight of chick; Inoculated when 12 days old.)

Chick No.	Day of the infection									
	1	2	3	4	5	6	7	8	9	10
1454	1.36	2.00	2.44	13.00	19.33	41.00	48.50*	30.00	.67	.00
1455	1.13	3.11	3.38	10.20	21.50*	18.00	18.00	.28	.00	.33
1456	1.12	2.12	2.31	Dead						
1457	Dead									
1458	1.43	1.46	1.50	6.58	16.66	16.66	17.50*	7.14	.00	.00
1459	2.11	4.63	6.60	15.25	25.50*	19.21	7.86	Dead		
1460	1.32	2.54	3.33	17.57	32.00	34.50*	16.66	4.80	.00	.00
1461	.93	1.11	4.55	11.36	25.50	26.50	52.00*	48.00	12.50	.93
1462	1.14	3.13	5.15	10.00	24.50	37.50	17.00	45.50*	23.00	Dead
1463	1.21	2.00	5.44	16.66	45.00	59.00*	35.00	9.20	.36	.33
Mean	1.31	2.46	3.86	12.62	26.24	31.55*	26.57	20.70	5.22	.27

*Day of highest parasitemia.

Table 2. Percentage of parasitized red blood cells present in infected 1 B series chicks during the ten-day period following inoculation with blood stages of P. lophurae from a passage chick. (Parasite dosage, 0.8×10^6 parasitized cells per 100 grams body weight of chick; inoculated when 12 days old.)

Chick No.	Day of the infection									
	1	2	3	4	5	6	7	8	9	10
1464	.83	1.86	3.00	9.09	17.33	27.50	29.00*	19.70	9.03	.47
1465	1.39	2.11	2.15	8.50	19.33	22.00*	18.66	10.00	Dead	
1466	2.12	2.16	3.01	10.52	18.00	34.50	20.00	38.00*	6.62	3.13
1467	1.13	1.39	2.27	5.50	17.33	36.50*	24.33	1.70	.53	.22
1468	2.00	2.04	2.94	15.50	18.86	44.50	57.00*	14.70	4.33	1.12
Mean	1.49	1.91	2.67	9.82	18.17	33.00*	29.80	16.82	5.13	1.24

*Day of highest parasitemia.

Table 3. Percentage of parasitized red blood cells present in infected 2 A series chicks during the ten-day period following inoculation with blood stages of P. lophurae from a passage chick. (Parasite dosage, 0.8 x 10^8 parasitized cells per 100 grams body weight of chick; inoculated when 12 days old.)

Chick No.	Day of the infection									
	1	2	3	4	5	6	7	8	9	10
1617	Dead									
1618	Dead									
1619	2.02	10.88	15.00	54.16	57.73*	52.00	Dead			
1620	Dead									
1621	Dead									
1622	3.06	11.37	7.50	50.28*	38.33	21.66	7.00	6.25	5.89	2.94
1623	Dead									
1624	4.17	13.00	17.50	38.67*	Dead					
1625	Dead									
1626	3.53	13.86	17.87	54.67	53.33	56.00*	32.33	15.00	9.83	3.64
Mean	3.20	12.28	14.47	49.45	49.66*	43.29	19.66	10.63	7.86	3.29

*Day of highest parasitemia.

Table 4. Percentage of parasitized red blood cells present in infected 3 A series chicks during the ten-day period following inoculation with blood stages of P. lophurae from a passage chick. (Parasite dosage, 0.8 x 10^6 parasitized cells per 100 grams body weight of chick; inoculated when 13 days old.)

Chick No.	Day of the infection									
	1	2	3	4	5	6	7	8	9	10
1681	3.87	6.21	25.33	27.00	35.85*	13.14	.00	.00	.00	.00
1682	3.03	6.92	25.74	32.22	68.00*	45.45	8.33	3.50	1.60	.50
1683	4.07	7.73	29.71	63.21	72.00*	Dead				
1684	5.65	6.90	42.50	51.00	57.14*	38.51	3.34	.97	2.33	.13
1685	3.05	6.23	20.23	22.60	58.76*	14.70	.00	.03	.00	.00
1686	4.97	9.96	41.66	52.63	100.00*	Dead				
1687	6.08	7.45	32.00	52.00	90.00*	43.47	9.28	1.93	.93	.40
1688	5.44	9.39	32.35	49.02	69.33*	24.14	1.00	.37	.27	.10
1689	4.34	13.20	36.00	48.17	48.57*	Dead				
1690	4.17	6.71	35.55	68.29*	62.35	Dead				
1691	3.88	6.78	27.58	35.17	61.05*	34.48	2.73	.13	.00	.00
1692	3.76	8.21	38.00	39.28	79.36*	37.14	6.70	.93	.43	.17
Mean	4.41	7.97	33.05	45.05	66.87*	31.88	3.92	.99	.70	.16

*Day of highest parasitemia.

Table 5. Percentage of parasitized red blood cells present in infected 4 A series chicks during the ten-day period following inoculation with blood stages of P. lophurae from a passage chick. (Parasite dosage, 0.8 x 10^8 parasitized cells per 100 grams body weight of chick; inoculated when 14 days old.)

Chick No.	Day of the infection									
	1	2	3	4	5	6	7	8	9	10
1840	2.64	5.77	22.61	37.00	49.50*	26.75	2.67	3.46	.57	.27
1841	2.87	5.55	9.37	21.50	38.50	62.20*	Dead			
1842	3.14	5.66	18.62	24.50	52.51*	31.00	3.33	.50	.00	.03
1843	2.17	6.75	21.38	32.50	45.77*	39.09	6.22	1.53	.33	.03
1844	3.34	5.53	28.13	39.00	58.50*	42.50	5.70	1.50	.67	.53
1845	6.83	8.33	30.01	36.50	56.50*	54.00	1.87	1.88	.60	.33
1847	2.00	1.73	19.48	34.00	48.00	52.50*	2.63	5.40	1.27	.10
1848	4.14	6.37	23.16	41.50	61.00*	55.00	6.50	.27	.27	.33
1849	6.00	6.25	10.43	30.50	35.00*	18.18	5.00	.63	.53	.47
1850	1.32	6.62	16.27	28.00	44.50*	39.50	6.23	.33	.07	.07
1851	3.33	7.28	24.31	39.00	58.50	67.20*	Dead			
1852	4.58	10.09	16.63	22.50	28.00	26.00	35.00*	34.50	29.33	16.67
1853	8.39	11.00	39.38	45.50	68.50*	45.50	22.67	2.65	1.13	.43
1854	7.36	9.16	26.36	31.50	53.00*	34.50	30.67	6.62	1.20	.37
1855	6.16	8.50	10.63	29.00	31.50	34.00*	10.60	3.58	1.93	Dead
Mean	4.28	6.97	21.12	32.80	48.61*	41.86	10.7	4.83	2.92	1.64

*Day of highest parasitemia.

Table 6. Percentage of parasitized red blood cells present in infected 5 A series chicks during the ten-day period following inoculation with blood stages of P. lophurae from a passage chick. (Parasite dosage, 0.8 x 10^8 parasitized cells per 100 grams body weight of chick; inoculated when 12 days old.)

Chick No.	Day of the infection									
	1	2	3	4	5	6	7	8	9	10
1	1.84	1.23	6.77	12.75	18.91	14.50	22.22*	16.66	5.00	.43
2	.81	.36	2.04	5.55	8.69	12.50	20.50*	20.18	13.25	1.11
3	1.13	1.23	6.55	11.16	13.60*	8.87	7. 28	.96	.00	.00
4	.89	1.23	3.57	10.80	18.18	28.00	35.00*	18.76	8.00	1.13
5	.91	.93	3.81	7.76	18.19	16.66	19.71*	4.72	.13	.00
6	.67	.83	4.50	14.10	22.50	34.00*	26.00	11.11	.26	.13
7	.62	.90	4.25	16.66	33.00	27.50	54.50	60.00	61.50*	58.50
8	1.11	1.33	5.66	13.00	20.80	25.50	39.00*	6.70	5.20	15.14
9	.82	1.20	5.10	7.45	8.16	6.45	22.22*	.23	.10	.00
10	1.13	1.40	7.63	17.00	21.20	32.00*	.96	6.25	17.00	12.15
Mean	.86	1.06	4.99	11.62	18.32	20.60	24.24*	14.56	11.04	8.91

*Day of highest parasitemia.

Table 7. Percentage of parasitized red blood cells present in infected 5 B series chicks during the ten-day period following inoculation with blood stages of P. lophurae from a passage chick. (Parasite dosage, 0.8×10^8 parasitized cells per 100 grams body weight of chick; inoculated when 12 days old.)

Chick No.	Day of the infection									
	1	2	3	4	5	6	7	8	9	10
11	.46	1.20	5.08	18.66	22.00	30.00*	24.00	24.40	21.09	23.55
12	.82	.96	6.60	5.66	11.58	11.18	10.00	12.75*	8.00	3.57
13	.74	.76	4.90	12.22	24.44	39.50	45.00*	22.22	24.04	39.00
14	.63	.63	4.15	11.37	25.33	20.40	28.00	42.50	56.00	57.00*
15	.99	1.36	5.09	10.60	19.00	36.50*	27.50	18.18	16.88	9.51
16	1.00	1.26	4.75	13.00	19.00	26.00	45.50*	41.50	41.50	43.00
17	.97	1.30	4.76	10.73*	Dead					
18	.79	1.00	2.79	8.87	12.00	18.18	5.67	11.11	9.27	26.50*
19	1.18	1.20	3.13	6.30	10.94	20.00	15.38	18.66	22.66	23.20*
20	1.01	1.06	4.91	9.63	12.50	16.66	18.18	31.50*	5.00	.30
Mean	.86	1.07	4.61	10.70	17.42	24.26	24.35	24.75*	22.72	22.56

*Day of highest parasitemia.

Table 8. Percentage of parasitized red blood cells present in infected 6 A series chicks during the ten-day period following inoculation with blood stages of P. lophurae from a passage chick. (Parasite dosage, 0.8×10^8 parasitized cells per 100 grams body weight of chick; inoculated when 13 days old.)

Chick No.	Day of the infection									
	1	2	3	4	5	6	7	8	9	10
1226	7.11	8.83	27.00	51.40	52.00	57.50*	38.50	36.00	26.00	Dead
1227	5.20	8.50	20.00	37.00	54.00	57.60*	41.50	31.00	Dead	
1228	4.63	12.75	29.00	57.50	66.50*	35.40	15.14	15.38	8.00	2.00
1230	3.78	11.60	26.00	64.00	71.50*	59.60	Dead			
1233	5.00	8.85	21.60	59.00	85.00*	55.50	30.00	Dead		
1234	3.78	10.60	26.50	63.00*	63.00*	45.50	36.00	Dead		
1235	6.37	18.33	37.50	61.00	67.00*	57.00	61.00	59.00	49.50	Dead
1236	3.33	9.66	25.00	64.00	71.00*	43.00	29.00	39.00	52.50	26.00
1237	4.63	11.20	28.50	73.50*	Dead					
1238	4.58	10.60	24.88	62.50	72.00*	49.50	25.00	24.44	9.33	5.20
Mean	4.84	11.09	26.60	59.29	66.94	51.17	34.52	34.14	29.07	11.07

*Day of highest parasitemia.

Table 9. Percentage of parasitized red blood cells present in ducks inoculated with phanerozoites of P. lophurae from two infected turkeys. Results include the period from the twelfth day following inoculation until all the ducks were sacrificed. (Inoculated when 5 days old.)

Duck No.	Day of the infection									
	12	13	14	15	16	17	18	19	20	21
40	Dead									
41	.00	Dead								
42	.00	.03	Dead							
43	.00	.00	.00	.10	.10	Dead				
44	.00	.00	.00	.10	Dead					
45	.00	.00	.00	.27	.43	.73	Dead			
46	.00	.00	.00	.03	.23	.80	5.67	Dead		
47	.00	.00	.00	.07	.13	.27	.00	.00	Dead	
49	.00	.00	.00	.00	.00	.00	.00	.00	.00	Dead
50	.00	.00	.00	.20	.83	.73	.13	.00	.00	.00
Mean	.00	.008	.00	.11	.29	.51	1.45	.00	.00	.00

Table 10. Temperature changes recorded in degrees Fahrenheit for 1 A series chicks on date of inoculation with blood stages of P. lophurae and for the ten-day period following inoculation.

Chick No.	Inoc. date	Day of the infection									
		1	2	3	4	5	6	7	8	9	10
1454	107.6	107.4	107.6	107.6	107.7	108.1*	106.7	106.9	107.6	107.6	107.4
1455	107.0	106.8	107.0	107.3	107.9*	106.4	106.8	107.0	107.0	107.1	106.8
1456	106.7*	106.5	106.0	105.3	Dead						
1457	107.4	Dead									
1458	107.0	106.7	106.6	106.8	107.6	108.0*	106.4	106.7	107.0	107.2	107.2
1459	107.0	106.8	107.0	107.1	108.6*	106.4	107.3	105.4	Dead		
1460	107.0	106.7	107.0	107.3	107.5	108.3*	107.6	107.4	107.5	107.2	107.3
1461	106.9	106.6	107.7	107.1	107.3	107.4	108.0*	105.8	106.7	107.0	106.9
1462	106.5	106.2	106.5	106.8	106.8	108.3*	106.0	106.3	106.3	104.1	Dead
1463	107.1	106.5	106.8	107.2	107.3	107.8*	105.9	106.5	106.8	106.7	106.9
Mean	107.0	106.7	106.8	106.9	107.6*	107.8*	106.8	106.5	107.0	106.7	107.1

*Day of highest temperature.

Table 11. Temperature changes recorded in degrees Fahrenheit for 1 B series chicks on date of inoculation with blood stages of P. lophurae and for the ten-day period following inoculation.

Chick No.	Inoc. date	Day of the infection									
		1	2	3	4	5	6	7	8	9	10
1464	106.5	106.3	106.6	106.6	106.7	107.0	107.8*	106.0	106.5	106.6	106.7
1465	107.5	107.1	107.3	107.4	107.7	108.1*	105.4	105.4	105.3	Dead	
1466	107.0	106.7	107.1	107.1	107.0	106.5	107.0	107.7*	106.7	106.3	105.8
1467	106.9	106.7	107.0	106.8	107.0	107.8*	106.0	106.5	106.9	107.0	106.9
1468	106.8	106.6	106.9	107.0	106.8	107.0	107.8*	105.9	106.9	107.0	106.8
Mean	106.9	106.7	107.0	107.0	107.0	107.3*	106.8	106.3	106.5	106.6	107.0

*Day of highest temperature.

Table 12. Temperature changes for non-inoculated 1 C series chicks for the same period of days as recorded for chicks of the 1 A and 1 B series.

Chick No.	Inoc. date	Day 1	2	3	4	5	6	7	8	9	10
1469	106.6	106.8	106.7	106.8	106.6	106.6	106.6	106.9	107.0	107.2*	106.9
1470	107.7	107.7	107.8	107.6	107.8	107.6	107.8	107.9*	107.6	107.6	107.7
1471	106.8	106.8	106.7	106.8	106.9*	106.9*	106.6	106.9*	106.8	106.8	106.7
Mean	107.0	107.1	107.1	107.1	107.1	107.0	107.0	107.3*	107.1	107.2	107.1

*Day of highest temperature.

Table 13. Temperature changes recorded in degrees Fahrenheit for 2 A series chicks on date of inoculation with blood stages of P. lophurae and for the ten-day period following inoculation.

Chick No.	Inoc. date	Day of the infection									
		1	2	3	4	5	6	7	8	9	10
1619	106.9	106.3	107.1	107.3	108.5*	107.4	106.1	Dead			
1622	107.5	107.0	107.6	108.1*	106.9	106.6	107.2	107.4	107.6	107.6	107.4
1624	107.4	107.1	107.3	107.6*	106.3	Dead					
1626	107.6	107.1	107.7	108.1*	107.4	107.9	107.1	107.4	107.4	107.6	107.4
Mean	107.3	106.9	107.4	108.0*	107.3	107.3	106.8	107.4	107.5	107.6	107.4

*Day of highest temperature.

Table 14. Temperature changes recorded in degrees Fahrenheit for 3 A series chicks on date of inoculation with blood stages of P. lophurae and for the ten-day period following inoculation.

Chick No.	Inoc. date	Day of the infection									
		1	2	3	4	5	6	7	8	9	10
1681	106.8	106.8	106.8	107.2	107.4	105.2	106.6	106.8	106.8	107.6*	107.0
1682	107.4	107.0	107.6	107.4	108.2*	106.4	106.0	106.4	106.8	107.4	107.4
1683	106.4	106.4	107.1	107.4	108.0*	106.6	Dead				
1684	107.0	106.4	107.5*	106.6	107.1	106.0	106.0	106.6	106.4	106.4	107.4
1685	107.4*	107.4*	106.8	107.2	107.2	106.6	105.8	107.0	106.8	107.0	107.4*
1686	106.6	106.2	107.0	107.8	108.4*	108.2	Dead				
1687	107.6	106.4	107.4	107.6	108.4*	106.3	106.1	106.4	107.0	107.2	106.0
1688	106.6	106.0	107.2	107.4	107.6*	105.5	105.8	105.6	105.8	106.8	Dead
1689	107.9	106.6	108.4*	107.8	107.7	106.5	Dead				
1690	107.6	107.6	107.6	107.8*	107.7	106.2	Dead				
1691	107.0	105.4	107.0	106.8	107.4*	106.8	106.8	107.0	107.4*	107.0	107.4*
1692	107.6	107.6	107.9	108.2	108.5*	106.4	106.6	107.1	108.4	107.5	108.4
Mean	107.2	106.7	107.4	107.4	107.8*	106.2	106.2	106.6	106.9	107.1	107.3

*Day of highest temperature.

Table 15. Temperature changes recorded in degrees Fahrenheit for 4 A series chicks on date of inoculation with blood stages of P. lophurae and for the ten-day period following inoculation.

Chick No.	Inoc. date	Day of the infection									
		1	2	3	4	5	6	7	8	9	10
1840	107.0	107.5	107.5	106.6	107.6*	106.8	106.4	106.6	106.4	107.2	107.0
1841	107.2	107.8	107.8	107.4	108.2*	107.2	107.6	Dead			
1842	107.4	107.4	107.6	107.6	108.2*	108.0	105.6	105.8	107.6	107.6	107.6
1843	107.6	107.4	107.4	107.5	107.8*	106.6	106.0	106.2	106.4	107.4	107.2
1844	106.6	107.0	107.0	108.0	108.2*	106.8	105.6	105.6	106.2	105.4	107.4
1845	107.0	107.4	107.4	108.0	108.2*	107.6	106.4	106.6	107.4	107.4	107.2
1847	107.0	107.6	107.6	108.4	108.5	108.2	108.6	107.0	107.0	108.2	107.2
1848	107.0	107.6	108.0	108.0	108.4*	106.8	105.4	106.4	106.6	107.6	107.8
1849	107.4	107.4	107.8	107.6	107.6	107.6	106.6	106.0	107.0	107.2	109.0*
1850	107.4	107.4	107.2	108.4	108.6*	107.8	107.0	107.0	107.4	107.4	108.0
1851	106.8	107.2	107.1	108.2	108.6*	107.6	106.8	Dead			
1852	106.6	107.0	107.0	106.6	107.6*	106.6	106.0	105.4	106.0	103.0	102.0
1853	107.0	107.6	107.4	108.0	108.1*	107.6	107.0	106.0	107.0	107.4	107.6
1854	106.8	106.8	107.0	107.1	107.6	108.4*	106.4	105.8	106.0	106.6	107.6
1855	106.4	106.4	106.7	107.0	107.8*	106.4	105.6	105.9	105.6	Dead	
Mean	107.0	107.4	107.4	107.6	108.1*	107.4	106.5	106.2	106.7	106.9	107.1

*Day of highest temperature.

Table 16. Temperature changes recorded in degrees Fahrenheit for 5 A series chicks on date of inoculation with blood stages of P. lophurae and for the ten-day period following inoculation.

Chick No.	Inoc. date	Day of the infection									
		1	2	3	4	5	6	7	8	9	10
1	107.0	107.0	107.2	107.0	106.4	107.7*	107.0	106.9	107.4	107.4	107.0
2	107.0	106.8	107.0	108.3	108.3	108.8*	108.6	107.0	107.4	107.6	106.8
3	107.4	107.4	107.4	107.1	107.0	107.8	107.4	106.8	108.0*	107.6	107.6
4	107.4	107.0	107.4	106.4	107.0	108.2*	107.4	107.2	107.2	107.0	107.0
5	107.4	107.2	107.4	106.3	108.0	108.6	108.8*	107.2	107.2	107.2	107.6
6	106.8	106.4	107.4	106.4	108.0	108.4*	107.2	105.8	106.8	107.4	108.0
7	106.6	106.8	107.2	107.0	108.2	108.6*	108.6*	107.6	107.4	106.6	106.2
8	107.0	106.8	106.6	106.4	106.2	107.1	107.2*	106.2	106.0	105.6	105.2
9	107.0	107.2	107.4	107.0	107.2	108.1*	107.6	107.4	107.6	107.4	107.8
10	106.5	106.6	107.4	106.3	107.2	107.6	106.8	107.8*	107.6	107.4	106.6
Mean	107.0	106.9	107.2	106.8	107.3	108.1*	107.7	107.0	107.3	107.1	107.0

*Day of highest temperature.

Table 17. Temperature changes recorded in degrees Fahrenheit for 5 B series chicks on date of inoculation with blood stages of P. lophurae and for the ten-day period following inoculation.

Chick No.	Inoc. date	Day of the infection									
		1	2	3	4	5	6	7	8	9	10
11	107.5	106.2	107.6*	107.1	107.1	107.6*	105.8	105.0	106.0	105.6	105.0
12	107.0	107.0	107.0	106.2	108.3*	108.3*	107.9	107.0	107.2	107.0	107.0
13	107.2	107.0	98.6	106.3	107.0	107.6*	107.1	106.2	107.0	106.2	106.6
14	107.5	107.3	107.4	107.2	107.3	108.2*	108.2*	107.2	107.0	107.2	106.6
15	107.0	106.2	106.6	107.2	108.1*	107.2	107.2	106.9	107.4	107.0	107.2
16	107.0	106.6	106.6	107.4	108.2	108.4*	107.4	106.0	106.5	106.4	104.8
17	106.6	106.4	106.6	106.3	108.0*	Dead					
18	106.6	106.8	106.6	106.3	106.4	107.4*	107.4*	106.0	107.0	106.8	106.0
19	107.0	107.0	106.8	107.0	107.2	106.6	107.7	106.7	107.6	108.0*	107.4
20	107.0	106.4	107.0	106.3	107.1	107.6*	107.6*	105.9	106.4	107.0	106.4
Mean	107.1	106.7	106.1	106.7	107.5	107.7*	107.4	106.4	106.9	106.8	106.3

*Day of highest temperature.

Table 18. Temperature changes recorded in degrees Fahrenheit for 6 A series chicks on date of inoculation with blood stages of P. lophurae and for the ten-day period following inoculation.

Chick No.	Inoc. date	Day of the infection									
		1	2	3	4	5	6	7	8	9	10
1226	106.4	105.6	106.6	107.8*	107.4	107.4	104.6	104.6	97.0	98.0	Dead
1227	106.4	103.6	106.6	107.8*	106.6	106.6	104.0	101.8	101.2	Dead	
1228	107.6	105.8	107.0	108.0	107.8	107.2	106.6	108.8	107.6	109.0	109.4*
1230	106.4	105.6	107.2	107.8	108.4*	107.6	105.6	Dead			
1233	106.6	106.4	106.4	107.4	107.6*	107.0	101.4	99.0	Dead		
1234	106.6	102.8	106.2	108.2*	108.0	105.6	103.4	96.6	Dead		
1235	106.6	104.6	106.6	106.6	107.6*	106.6	106.4	105.6	102.6	98.6	Dead
1236	106.4	103.8	106.6	108.6*	107.6	107.6	106.4	104.6	105.4	107.4	105.4
1237	105.8	104.0	106.8*	106.6	Dead						
1238	106.6	104.6	107.4	107.6	108.2*	106.0	105.0	105.0	104.2	107.0	107.0
Mean	106.5	106.7	106.7	107.6	107.7*	106.9	104.8	103.3	103.0	104.0	107.3

*Day of highest temperature.

Table 19. Temperature changes recorded in degrees Fahrenheit for 6 B series chicks on date of inoculation with blood stages of P. lophurae and for the ten-day period following inoculation.

Chicks No.	Inoc. date	Day of the infection									
		1	2	3	4	5	6	7	8	9	10
1229	105.2	102.0	103.0	106.4	105.8	107.0*	105.8	104.0	99.8	101.0	Dead
1231	106.2	106.2	106.2	107.0	107.2*	107.2*	106.6	106.5	106.0	106.7	106.6
1232	106.8	107.1	106.9	107.0	106.8	106.8	106.7	106.9	107.0	107.3*	106.8
Mean	106.1	105.1	105.4	106.8	106.6	107.0*	106.4	105.8	104.3	105.0	106.7

*Day of highest temperature.

Table 20. Daily weight changes recorded in grams for 1 A series chicks on the inoculation date with blood stages of P. lophurae through the ten-day period following inoculation. (Diet given the chicks was the G-ration; age when placed on the G-ration, 5 days).

Chick No.	Inoc. date	Day of the infection									
		1	2	3	4	5	6	7	8	9	10
1454	70	67	81	79	88	93	95	104	95	102	108
1455	77	74	84	85	99	104	103	103	110	117	128
1456	77	79	86	79	Dead						
1457	Dead										
1458	70	68	81	81	92	95	98	94	96	107	119
1459	93	87	95	94	104	110	105	93	Dead		
1460	71	73	77	79	88	94	90	85	80	87	88
1461	82	79	86	89	88	88	85	93	86	84	95
1462	60	57	60	63	68	68	70	70	70	69	Dead
1463	93	93	83	83	98	100	101	85	95	108	107
Mean	77	75.2	81.4	82.8	90.6	94	93.4	91	90.2	96.3	107.5

Table 21. Daily weight changes recorded in grams for 1 B series chicks on the inoculation date with blood stages of P. lophurae through the ten-day period following inoculation. (Diet given the chicks was regular chick starting ration for the period throughout the investigation).

Chick No.	Inoc. date	Day of the infection									
		1	2	3	4	5	6	7	8	9	10
1464	64	69	72	72	79	82	82	85	85	87	94
1465	60	56	61	62	66	68	66	60	55	Dead	
1466	64	62	65	68	76	80	81	78	78	83	92
1467	78	80	87	88	91	94	99	104	107	110	117
1468	69	73	82	85	93	99	98	96	95	104	113
Mean	67	68	73.4	75	81	84.6	85.2	84.6	84	96	104

Table 22. Daily weight changes recorded in grams for non-inoculated 1 C series chicks for the same period of time as that for chicks of the 1 A and the 1 B series. (Diet given the chicks was the regular chick starting ration for the period throughout the investigation).

Chick No.	Inoc. date	Day of the infection									
		1	2	3	4	5	6	7	8	9	10
1469	64	63	69	73	80	85	90	97	106	112	119
1470	70	73	80	83	88	96	105	111	115	120	127
1471	73	74	81	85	94	101	108	116	118	125	129
Mean	69	70	77	80	87	94	101	108	114	119	125

Table 23. Daily weight changes recorded in grams for 2 A series chicks on the inoculation date with blood stages of P. lophurae through the ten-day period following inoculation. (Diet given the chicks was the G-ration; age when placed on the G-ration, 3 days).

Chick No.	Inoc. date	Day of the infection									
		1	2	3	4	5	6	7	8	9	10
1617	Dead										
1618	Dead										
1619	85	80	86	103	112	100	92	Dead			
1620	82										
1621	Dead										
1622	76	68	83	93	91	93	83	89	95	105	119
1623	Dead										
1624	54	51	57	65	65	Dead					
1625	Dead										
1626	75	63	75	94	103	105	90	95	104	111	123
Mean	74.4	65.5	75.3	88.8	92.8	99.3	88.3	92	99.5	108	121

Table 24. Daily weight changes recorded in grams for 3 A chicks on the inoculation date with blood stages of P. lophurae through the ten-day period following inoculation. (Diet given the chicks was the G-ration; age when placed on the G-ration, 5 days).

Chick No,	Inoc. date	Day of the infection									
		1	2	3	4	5	6	7	8	9	10
1681	93	93	107	115	110	104	91	112	105	110	120
1682	90	87	103	108	115	110	98	112	110	120	123
1683	103	102	118	120	123	120	Dead				
1684	100	100	117	122	127	114	104	117	118	117	128
1685	77	78	94	94	93	95	98	104	102	108	109
1686	93	90	105	107	110	108	Dead				
1687	101	95	117	118	125	124	111	123	121	135	133
1688	90	93	112	113	113	105	95	114	115	118	132
1689	79	81	89	96	101	100	Dead				
1690	99	97	104	113	112	103	Dead				
1691	90	81	92	96	96	98	103	113	108	116	124
1692	98	94	107	114	118	103	91	110	105	115	112
Mean	92.8	90.9	105.4	109.7	111.9	107.8	98.9	113.1	110.5	117.4	122.6

Table 25. Daily weight changes recorded in grams for 4 A series chicks on the inoculation date with blood stages of P. lophurae through the ten-day period following inoculation. (Diet given the chicks was the Q-ration; age when placed on the Q-ration, 5 days).

Chick No.	Inoc. date	Day of the infection									
		1	2	3	4	5	6	7	8	9	10
1840	67	72	78	77	79	76	74	74	77	84	88
1841	72	72	71	77	76	75	74	Dead			
1842	85	93	104	105	102	107	99	96	105	114	122
1843	102	104	98	103	100	95	93	85	101	107	114
1844	82	85	90	97	91	86	80	85	88	88	95
1845	76	80	87	90	88	91	85	86	88	87	87
1847	85	91	90	94	89	95	93	95	98	107	114
1848	79	85	95	96	93	85	77	84	85	89	90
1849	66	69	80	83	80	80	79	78	76	80	83
1850	97	110	114	118	115	110	108	110	116	123	134
1851	92	102	112	112	108	108	100	Dead			
1852	63	70	65	67	61	64	63	63	63	66	70
1853	101	105	113	113	109	110	105	105	104	114	127
1854	67	70	74	80	75	75	72	74	74	80	85
1855	70	69	77	75	71	71	67	63	59	Dead	
Mean	80.3	85.2	89.9	92.5	89.1	88.5	84.6	85.2	87.2	94.9	100.8

Table 26. Daily weight changes recorded in grams for 5 A series chicks on the inoculation date with blood stages of P. lophurae through the ten-day period following inoculation. (Diet given the chicks was the R-ration; age when placed on the R-ration, 5 days).

Chick No.	Inoc. date	Day of the infection									
		1	2	3	4	5	6	7	8	9	10
1	86	90	95	103	103	105	110	117	120	120	129
2	87	90	96	105	102	102	105	109	110	116	113
3	82	88	95	101	106	104	104	105	109	115	121
4	81	83	88	93	96	97	95	98	98	102	103
5	90	93	98	102	103	100	104	116	105	109	115
6	79	82	85	91	89	90	90	90	92	96	99
7	82	85	90	94	98	96	100	103	104	108	105
8	84	88	92	100	104	108	110	107	112	115	120
9	118	110	118	123	130	128	125	133	138	143	147
10	78	83	85	93	94	93	90	90	94	100	108
Mean	86.7	89.2	94.2	100.5	102.5	102.3	103.3	106.8	108.2	111.8	116

Table 27. Daily weight changes recorded in grams for 5 B series chicks on the inoculation date with blood stages of P. lophurae through the ten-day period following inoculation. (Diet given the chicks was the Q-ration; age when placed on the Q-ration, 5 days).

Chick No.	Inoc. date	Day of the infection									
		1	2	3	4	5	6	7	8	9	10
11	81	83	88	89	91	88	84	83	78	75	73
12	85	89	95	97	100	103	103	103	105	95	97
13	78	82	85	81	86	85	85	82	85	85	84
14	90	92	98	102	117	108	107	108	107	107	105
15	79	85	89	93	95	96	92	95	98	103	105
16	75	76	79	82	85	87	83	84	85	88	88
17	78	84	88	95	92	Dead					
18	80	85	93	103	105	105	105	105	104	105	103
19	88	92	96	105	117	97	97	98	98	98	96
20	93	95	100	105	109	109	109	109	113	118	110
Mean	82.7	86.3	91.1	99.2	99.7	97.6	96.1	96.3	97.0	97.1	95.7

Table 28. Daily weight changes recorded in grams for 6 A series chicks on the inoculation date with blood stages of P. lophurae through the ten-day period following inoculation. (Diet given the chicks was the S-ration, age when placed on the S-ration, 5 days).

Chick No.	Inoc. date	Day of the infection									
		1	2	3	4	5	6	7	8	9	10
1226	80	84	88	90	96	95	96	90	85	80	Dead
1227	79	82	88	85	88	94	93	92	87	Dead	
1228	81	86	86	90	93	88	91	92	93	99	106
1230	80	82	88	88	92	90	85	Dead			
1233	82	88	93	90	89	85	76	69	Dead		
1234	72	75	78	80	83	78	78	77	Dead		
1235	69	78	69	70	73	70	65	65	64	59	Dead
1236	77	83	88	84	83	85	85	90	93	98	103
1237	89	88	91	90	93	Dead					
1238	90	93	98	102	107	98	88	90	87	95	98
Mean	79.9	83.9	96.7	86.9	89.7	87	83.4	83.1	84.8	86.2	102.5

Table 29. Daily weight changes recorded in grams for non-inoculated 6 B series chicks for the same period of time as that for chicks of the 6 A series. (Diet given the chicks was the S-ration; age when placed on the S-ration, 5 days).
Days indicated correspond to days of infection of 6 A series.

Chick No.	Inoc. date	Day 1	2	3	4	5	6	7	8	9	10
1229	56	58	57	60	60	67	65	67	67	Dead	
1231	107	108	112	119	127	137	140	158	168	178	193
1232	82	89	94	100	109	111	115	124	133	142	151
Mean	82	85	88	93	99	105	107	116	123	160	172

Table 30. Weight changes recorded in grams for ducks on the day before inoculation with phanerozoite stages of P. lophurae until 15 days old. (Diet given the ducks was the regular chick starting ration for the period throughout the investigation).

Duck No.	Day before inoc.	Inoc. date	Day of the infection						
			1	4	6	8	11	13	15
40	54	60	68	105	148	194	Dead		
41	54	57	64	97	133	170	245	Dead	
42	57	63	71	90	113	148	215	230	Dead
43	58	66	72	105	150	193	280	315	410
44	58	60	65	94	120	138	168	285	230
45	53	67	75	112	163	207	310	343	435
46	56	69	80	130	183	248	385	420	500 +
47	62	70	86	134	183	235	310	384	500 +
49	52	64	75	90	117	145	220	230	350
50	56	61	74	107	147	190	280	290	390
Mean	56	63.7	73	106.4	145.7	186.8	268.1	312.1	402.1

Table 31. Microscopic examination of various organs.

Organs examined	Number examined			Phanerozoites			Pigment and phagocytosis		
	Chick	Duck	Turkey	Chick	Duck	Turkey	Chick	Duck	Turkey
Adrenals	25	6	2	0	0	0	13	1	0
Bone marrow	15	3	0	0	0	0	1	0	0
Brain	48	10	2	0	0	1	30	2	1
Eyelid	42	0	0	0	0	0	10	0	0
Gizzard	5	0	0	0	0	0	0	0	0
Gonad	32	10	2	0	0	0	13	0	0
Heart	28	10	2	0	0	0	3	0	0
Intestine	10	0	0	0	0	0	1	0	0
Kidney	50	10	2	0	0	0	36	4	1
Liver	50	10	2	0	0	0	46	7	2
Lung	45	10	2	0	0	0	36	2	1
Pancreas	15	10	2	0	0	0	9	6	0
Rectum	5	0	0	0	0	0	0	0	0
Spleen	50	10	2	0	0	0	48	6	2
Total	417	89	18	0	0	1	246	27	7

Table 32. The appearance of eyelid lesions in chicks fed on different rations.

Series No.	Group	Ration*	No. in group	No. with eyelid lesions	Mean percent** 4th day	5th day	6th day	7th day
1	1 A	G	10	4	12.6	26.2	31.6	26.6
	1 B	B	5	0	9.8	18.2	33.0	29.8
	1 C	B	3	0	not inoculated			
2	2 A	G	10	0	49.5	49.7	43.1	19.7
3	3 A	G	12	0	45.1	66.9	31.9	3.9
4	4 A	Q	15	3 (slight)	32.8	48.6	41.9	10.7
5	5 A	R	10	0	11.6	19.3	20.6	24.2
	5 B	Q	10	0	10.7	17.4	24.3	24.4
6	6 A	S	10	0	59.3	66.9	51.2	34.5
	6 B	S	3	0	not inoculated			

* B = basic or standard chick diet
G = G-ration
Q = Q-ration
R = R-ration
S = S-ration

** Mean percent of parasitized red blood cells on each day indicated.

Table 33. Mean weights of chicks given different diets to show comparisons on date of inoculation and tenth day following inoculation. (Weight in grams)

Series number	Ration given chicks	Mean group weight, inoc. date	Rank among all groups according to weight	Mean group wt. 10 days after inoc.	Rank among all groups according to weight	Mean gain in 10 days	Rank among all groups according to weight
1 A	G	77.0	7	107.5	6	30.5	5
1 B	B	67.0	10	104.0	7	37.0	4
1 C	B	69.0	9	125.0	2	56.0	2
2 A	G	74.4	8	121.0	4	46.6	3
3 A	G	92.8	1	122.6	3	29.8	6
4 A	Q	80.3	5	100.8	9	20.5	9
5 A	R	86.7	2	116.0	5	29.3	7
5 B	Q	82.7	3	95.7	10	13.0	10
6 A	S	79.0	6	102.5	8	22.6	8
6 B	S	82.0	4	172.0	1	90.0	1
Mean		79.2		116.7		37.5	

Table 34. Comparisons of the various groups of chicks given different rations.

Series No.	1 A	1 B	1 C	2 A	3 A	4 A	5 A	5 B	6 A	6 B
No. of birds used	10	5	3	10	12	15	10	10	10	3
Diet given to group	G	Reg.	Reg.	G	G	Q	R	Q	S	S
Age of birds when placed on diet	5	1	1	3	5	5	5	5	5	1
Age of birds when inoc. with P. lophurae	12	12	not inoc.	12	13	14	12	12	13	not inoc.
Mean wt. in grams on day of inoculation	77	67	69	74.4	92.8	80.3	86.7	82.7	79.9	82
Mean gain over 10-day period	30.5	37	56	46.6	29.8	20.5	29.3	13.0	22.6	90
Mean wt. in grams on day 10 after inoculation	107.5	104.0	125.0	121.0	122.6	100.8	116.0	95.7	102.5	172.0
Day of highest mean parasitemia	6	6	-	5	5	5	7	8	5	-
Mean percent of parasitized red blood cells on day of highest mean parasitemia	31.55	33.00	-	49.66	66.87	48.61	24.24	24.75	69.94	-
Day on which highest mean temperature occurred	5	5	7	3	4	4	5	6	4	5
Mean temperature on day of highest mean temperature (degrees Fahrenheit)	107.7	107.9	107.2	107.8	107.8	108.1	108.1	107.6	107.7	107.1

Table 35. Further comparisons of chicks on different rations, and of ducks and turkeys.

Series No.	1 A	1 B	1 C	2 A	3 A	4 A	5 A	5 B	6 A	6 B	Ducks	Turkeys	Totals
No. examined for parasitized red blood cells	9	5	0	4	12	15	10	10	10	0	10	2	87
No. with parasitized red blood cells	9	5	0	4	12	15	10	10	10	0	7	2	84
No. examined for eyelid lesions	9	5	3	4	12	15	10	10	10	3	10	2	93
No. with eyelid lesions	4	0	0	0	0	3 (slight)	0	0	0	0	0	0	7
No. examined for phagocytosis	6	3	0	2	7	10	6	8	8	0	10	2	62
No. showing phagocytic activity	5	3	0	2	7	9	5	6	6	0	5	1	49
No. examined for malarial pigment	6	3	0	2	7	10	6	8	8	0	10	2	62
No. showing some pigment	6	3	0	2	7	10	6	8	8	0	7	2	59
No. examined for phanerozoites	6	3	0	2	7	10	6	8	8	0	10	2	62
No. with phanerozoites	0	0	0	0	0	0	0	0	0	0	0	1	1